THE FORMATION AND REACTIONS OF SULPHUR COMPOUNDS DURING DISTILLATION

by

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ABBREVIATIONS

Cu^{+}	cuprous ion
Cu^{2+}	cupric ion
DMS	dimethyl sulphide
DMDS	dimethyl disulphide
DMTS	dimethyl trisulphide
DMTeS	dimethyl tetrasulphide
EMS	ethyl methyl sulphide
FPD	flame photometric detector
GC	gas chromatography
SCD	sulfur chemiluminesence detector
$CuAc_2$	cupric acetate
EDTA	ethylendiamintetraacetic acid disodium salt dihydrate

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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ABSTRACT

Due to their importance as flavour compounds, the mechanism of the formation and reactions of sulphur compounds, especially dimethyl disulphide (DMDS) and dimethyl trisulphide (DMTS), during malt whisky distillation and the factors which affect these compounds were investigated using a small scale distillation system with methional and methionine. DMDS and DMTS were both identified in reaction products from methional and methionine. The duration of heating, presence and amount of copper salts, anions or oxidant, aerobic conditions and pH affected the formation of these polysulphides from methional. It is suggested that DMDS is formed from methanethiol, which was formed from methional and L-methionine. DMTS appears to be formed from methanethiol and H_2S. This is the first time that formation of DMDS and DMTS from methional and methionine has been conclusively demonstrated under conditions that simulate malt whisky distillation.

1. INTRODUCTION

Flavour and aroma are very important in foods and beverages. These flavours and aroma compounds give beverages their typical odours and tastes, and are therefore one of the key attributes in determining the palatability of foods and beverages.

1.1 Flavour of Foods and Beverages

For many years, flavour researchers have been investigating why the flavours of certain products are attractive, what compounds are formed by cooking and what factors affect the formation of flavour compounds. As the result of efforts of these flavour researchers, the number of identified flavour compounds in foods and beverages increased from 500 to about 6300 between 1963 and 1993 (Boelens *et al.*, 1994).

Over 1300 volatile compounds have been identified in alcoholic beverages giving typical odour and taste (Nykänen, 1986). Table 1.1 shows the number of volatile compounds identified in distilled alcoholic beverages. These numbers should increase with improved analytical methods in the future. However, it is more important to know which compounds contribute to the quality of a product in positive or negative ways and to understand the formation mechanisms of these compounds. These volatile compounds can be classified into several groups according to their chemical nature. Fusel alcohols, fatty acids and esters form the largest group. Fewer flavour active carbonyl compounds, phenols, nitrogen compounds and sulphur compounds are known,

but their contribution to flavour can be greater than the major group, as they have very low flavour thresholds.

Many compounds are found in whisk(e)y, these compounds must give a whisk(e)y typical odour and taste. Materials and the production process of whisky can affect these compounds.

Table 1.1 Number of volatile compounds identified in distilled alcoholic beverages (Maarse and van den Berg, 1994)

Product	Number	Product	Number
Whisky		Grape brandy	
Scotch malt	227	Cognac	486
Scotch blended	184	Armagnac	77
Bourbon	127	German	181
Irish	88		
Canadian	86	Rum	550
Japanese	109		

1.2 Whisky Production

Whisk(e)y derives its name from the Gaelic *uisge-beatha* which means 'water of life'. Whiskies are popular spirits and are produced in many countries, especially Scotland, Ireland, Canada, USA and Japan, under their own regulations. The best known type is Scotch whisky blended of malt and grain whisky (EU, 1989). A comprehensive scientific explanation of all aspects of whisky production has recently been published (Piggott *et al.*, 1989). Therefore, it is not necessary to describe the whisky making process. However, the book was concerned with science and technology rather

than flavour research. The flavour compounds of whisky originate in the different production stages which include malting, mashing, fermentation, distillation and maturation. Recently, several surveys of the contributions of processing to whisky flavour have been published (Paterson and Piggott, 1989; Maarse and van den Berg, 1994; Cole and Noble, 1995).

1.2.1 Materials

Malted barley is the sole raw material for malt whisky; Maize or wheat hydrolysed by malted barley is used for grain whisky. At present, wheat is the preferred cereal in the Scotch grain whisky industry because of EU import duty on maize.

Originally, the process of malting of barley was carried out in each distillery. Peat was used for the kilning of the malt. During this process the germinated barley was exposed to peat smoke and heat. The Maillard reaction and pyrolysis result in the formation of phenols, pyrizines, pyrazines and thiazoles. These volatile compounds are transfered into the malt whisky spirit (Howie and Swan, 1984; Viro, 1984a; 1984b; Piggott *et al.*, 1993) and give a smoky and peaty character. Today, only a few distilleries have their own maltings since specialised maltsters can supply the distillery with malt made to their own specifications. The kilning process has a major effect on the formation of malt flavour compounds even if malt is lightly peated or non-peated (Paterson and Piggott, 1989; Maarse and van den Berg, 1994). However, barley quality also affects the whisky quality. Watson (1983a) reported that a higher nitrogen

level than normal resulted in a heavy and "fuselly" quality in spirits.

1.2.2 Mashing

Mashing is the process of extracting fermentable sugar into wort. There are two major mashing processes in the malt and grain whisky industries. Cooking of grain-water mixture produces sulphur compounds, such as H_2S, carbonyl sulphide, methanethiol, dimethyl sulphide (DMS) and dimethyl disulphide (DMDS) (Ronkainen, 1973; Buckee, 1982). Although some reactions which contribute to whisky spirit flavour must occur during the mashing process in a malt whisky distillery, they have not been mentioned in the literature. Shirakashi *et al.* (1995) reported that pH reduction of wort during mashing by *Bacillus coagulans* caused an increase of isoamyl acetate, β-phenethyl acetate and some sulphur containing compounds in whisky spirits.

1.2.3 Fermentation and Yeast

During the alcoholic fermentation, yeast (*Saccharomyces cerevisiae*) produces ethanol and carbon dioxide from sugars. Simultaneously yeast produces a number of by-products of higher alcohols, fatty acid and ethyl esters (Nykänen, 1986; Paterson and Piggott, 1989; Nykänen and Nykänen, 1991). Yeast and fermentation conditions such as temperature, pH, oxygen condition significantly affect the whisky flavour (Maarse and van den Berg, 1994). A number of malt distilleries use both distillers yeast and brewers yeast (Korhola *et al.*, 1989). Inoculum level (Berry and Ramsay, 1983) and yeast strains (Korhola *et al.*, 1989) significantly

affect on the total higher alcohols and fatty acid esters of wash. The effect of some *Lactobacillus* spp. on whisky flavour has been published (Barbour and Priest, 1988; Geddes and Riffkin, 1989; Makanjuola *et al.*, 1992).

1.2.4 Distillation

Traditional batch (pot) distillation and continuous (column) distillation systems are used to produce malt whisky and grain whisky, respectively. Most malt distilleries employ a double distillation system. Today, Irish distilleries and only one Scotch malt distillery employ a triple distillation system (Varnam and Sutherland, 1994). However, the second and third distillations are different in Ireland and Scotland. The type of distillation, double or triple, affects spirit flavour due to the degree of rectification (Watson, 1983a).

Undoubtedly, distillation is one of the most important steps to make whisky flavour characters. The distillation process is not only an extraction stage of volatile compounds from wash, but also a reaction stage in which the flavour compounds are formed from precursors or are reacted with other compounds by heating. The Maillard reactions, between amino acids and reducing sugars, can form heterocyclic compounds, such as furans, pyrroles, pyridines and especially pyrazines, during distillation (de Rijke and ter Heide, 1983). Other sulphur-containing compounds, such as thiophens and polysulphides, are formed during distillation by pyrolysis reaction (Maarse and van den Berg, 1994). Diethyl acetal is formed during distillation (Nykänen and Nykänen, 1983).

1.2.5 Batch Distillation

Although continuous distillation had been introduced and developed in the grain whisky industry, pot still distillation survived in malt whisky distilleries.

1.2.4.1 Pot Still Design

Traditionally, the pot still (Figure 1.1) and condenser are manufactured from copper. Firstly, copper had been chosen for its malleability and good heat conductivity. At the present time, copper has continued in use as the only suitable material for high quality malt whisky (Nicol, 1989). Copper is dissolved gradually into wash during wash distillation and the level of dissolved copper was reported to reach 15 μg/mL after distillation (Quinn *et al.*, 1981). Many different shapes of still are used in the malt whisky industry (Figure 1.2). Recently, it has been stated that mist transfer occurred during pot distillation, and this affected the quality of malt whisky spirits (Ohtake *et al.*, 1995). Therefore, the height of the swan neck and slope of the lyne arm affect this mist transfer, but a purifier in the lyne arm (Figure 1.3), which most still do not have, interrupts or reduces it. Most distilleries have changed their heating method from direct coal, peat or gas firing to steam heating by coil, plate, or external heater. Steam heating gives easy control of distillation, especially wash distillation, and a reduction of energy cost. Today only a few distilleries still use a direct firing system, which they believe adds a necessary character into their malt whisky spirits. Another change has taken place in the

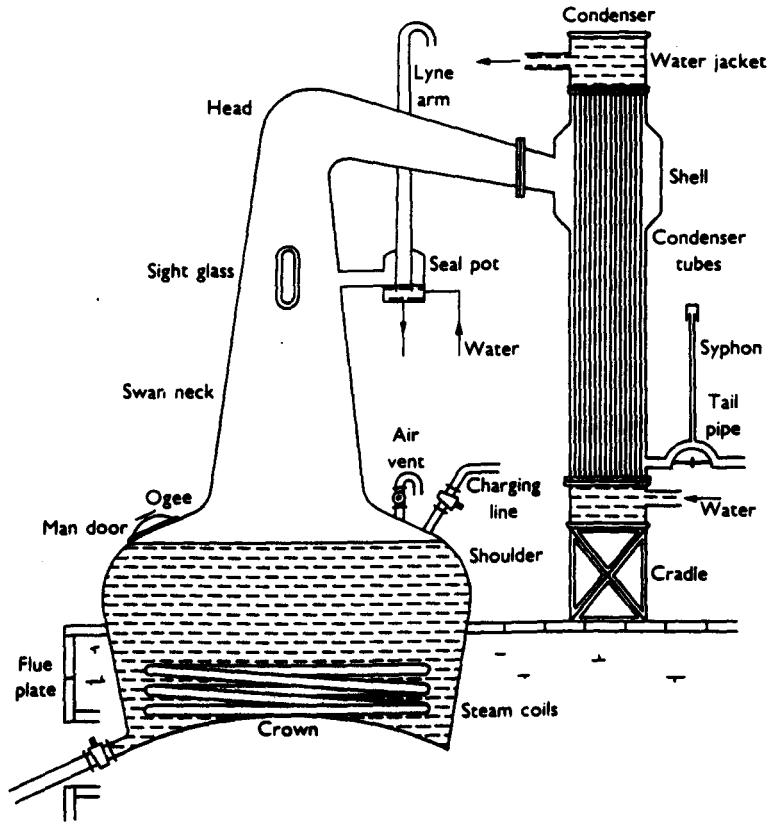

Figure 1.1 Wash still (Nicol, 1989)

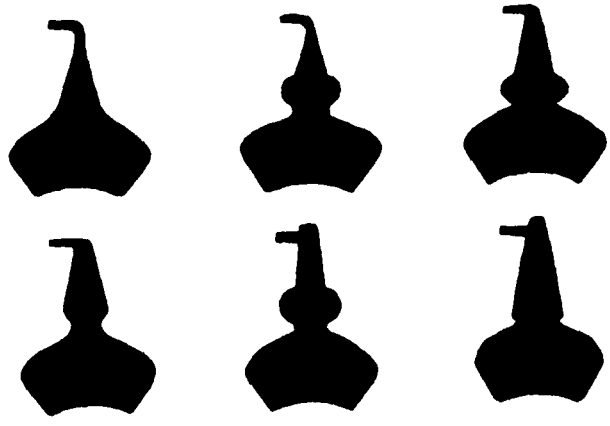

Figure 1.2 A selection of different pot still shapes (Whitby, 1992)

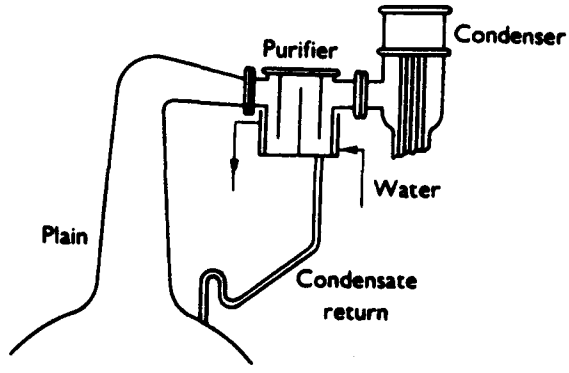

Figure 1.3 Purifier (Nicol, 1989)

condenser system, from traditional worm to the moreefficient shell and tube condenser.

1.2.4.2 Operation of Distillation

The traditional Scotch whisky pot distillation has two stages. The first and second distillation are called wash and spirit distillation, respectively.

The entire distillate of the wash still is collected as 'low wines'. Wash distillation is stopped when distillate strength at the safe is approximately 1% (v/v) ethanol, with the volume of low wines about one-third of the original charge into wash still. To prevent wash foaming into the condenser, distillation is controlled carefully by the stillman, especially in its initial stage of the wash distillation.

Three fractions, known as foreshots, spirit and feints, are taken from the spirit distillation. Foreshots and feints are returned to the spirit still with the next batch of low wines, and then are distilled. The timings of switching from

foreshots to spirit and from spirit to feints are critical in determining the final whisky spirit flavour. Fatty acid esters appear in the vapour in distillation and decrease quickly with time; higher alcohols appear later and then also decrease with time, whereas fatty acids and 2-phenyl ethanol appear late and increase with time (Cantagrel, 1989). Therefore, foreshots contain a lot of fatty acid ester, especially ethyl acetate, and higher alcohols, such as isoamyl alcohol. However, in a series of distillations, eventually the original amount of these compounds in the wash will be transferred into the spirit. The aim of foreshots is the separation of the undesirable high molecular compounds which are retained in the condenser and pipework from the feints of the previous batch (Paterson and Piggott, 1989). The common test is the mixing of equal volumes of distillate sample and water. The absence of cloudiness is regarded as determining the cut point (Paterson and Piggott, 1989). The cut to feints is determined by alcohol content in the distillate. This timing of cut point is significant for spirit quality, as it can effect the concentrations of fatty acids which increase with time during distillation (Cantagrel, 1989).

1.2.4.3 Reactions with Copper

Masuda and Nishimura (1981) reported that copper was involved in the formation of DMDS in wash distillation. When wash was distilled in glass, the DMDS concentration in low wines was about one tenth that in a copper still. Watson (1983b) reported that when copper wire was placed inside the still body, the still head and the condenser, the level of

most of the esters in spirit was increased. Copper apparently catalyses these esterification reactions. He also showed that in the absence of copper in both wash and spirit still, the spirit was harsh. This harshness was slightly reduced in the presence of copper in the wash still. When copper was placed in the spirit still alone, this harshness was further reduced.

Beveridge (1990) reported that the surface activity of the copper still was important because after parts of wash still were replaced at a commercial distillery, the level of DMTS in low wines was decreased. Whitby (1992) also reported a similar case that after the lyne arm (see Figure 1.1) was replaced due to wear at one commercial malt distillery, the sulphury note which is typical in this distillery, was absent. This was confirmed by decrease in the levels of DMTS in spirit.

1.2.6 Continuous Distillation

The continuous Stein still was introduced into the Scotch whisky industry in 1827, and later, Aeneas Coffey improved and patented it in 1830. This type of still, known as Coffey or patent still, is still used in the Scotch grain industry today. The Coffey still has two columns (Figure 1.4), 'analyser' and 'rectifier'. The cold wash is preheated through the rectifier and then fed into the top of analyser column. Steam is fed into the bottom of the analyser. In the column, distillation occurs on each plate, the condensed liquid flows down to the plate below but the steam causes the liquid to boil and vapour flows through the hole in the next higher plate, then vapour heats the liquid. The spent

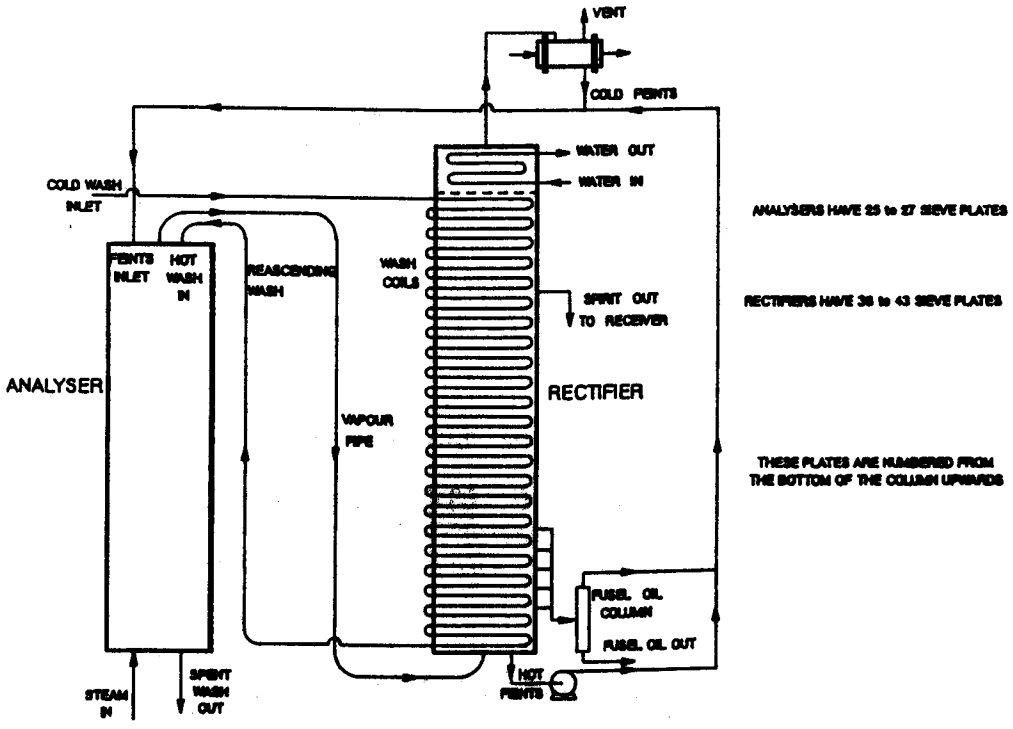

Figure 1.4 Coffey still (Whitby 1992).

wash which does not contain ethanol is removed from the bottom of analyser. The vapour from the top of analyser is introduced into the bottom of rectifier, while the feints from the bottom of rectifier are fed back into the top of the analyser. Recently, comprehensive reviews providing an overview of continuous distillation have been published (Panek and Boucher, 1989; Whitby, 1992; Nicol, 1993; Piggott and Conner, 1995).

In grain whisky production, copper plays an important role in removing or modifying the unpleasant flavour

compounds in the final spirit. Therefore, some copper must be present in the system, although some column still are now constructed of stainless steel (Whitby, 1992; Piggott and Conner, 1995). With the high rectification to 94% (v/v) ethanol, grain whiskies are of a light-flavour type.

1.2.7 Maturation

New make whiskies have unacceptable and unpleasant odours and a sharp taste. These characters can be modified by maturing the distillate in oak casks. Many comprehensive reviews of the chemical changes of whisky maturation have been published (Reazin, 1981; Reazin, 1983a; Reazin, 1983b; Sharp, 1983; Watson, 1985; Perry, 1986; Philp, 1986; Nishimura and Matsuyama, 1989; Nykänen and Nykänen, 1991; Maarse and van den Berg, 1994; Cole and Noble, 1995; Piggott and Conner, 1995; Piggott *et al.*, 1995).

The changes in the compounds during maturation can be divided into the following types:

- Subtractive maturation

  Evaporation

  Adsorption on carbon of charred oak

  Oxidation (sulphur compounds, aliphatic carbonyls, pyridines)

- Additive Maturation

  Tannins (ellagitannins, gallic acid, ellagic acid)

  Lignin degradation compounds (vanillin, syringaldehyde, coniferaldehyde, sinapaldehyde)

  Hemicelluloses (glucose, arabinose, xylose, furfural, 5-hydroxymethyl furfural)

  Acetic acid

Maltol

Woody lactones

◆ Other changes

Esterification

These reaction changes are affected by the following factors:

Cask

Cask size (surface-to-volume ratio)

Wood type (eg. American or Spanish oak)

Porosity and thickness of wood

History of cask (number of prior uses of cask)

Cask treatment (charring, sherry seasoning)

Maturation condition

Filling strength of spirits

Duration of maturation

Temperature and relative humidity in the warehouse

Alcohol and oxygen concentrations in the warehouse

Agitation

The concentration changes of sulphur compounds during maturation are described in section 1.3.6.

1.3 Dimethyl Polysulphides

Many sulphur compounds identified in whisky are known to play an important role in the flavour quality of whisky due to their characteristic odour and low flavour thresholds (ter Heide, 1986). It has been established that DMS is a key flavour in beer. Much information on its derivation from malt and the control of its concentration in beer has been published (Niefind and Späth, 1974; White, 1977; Nakajima and Narziss, 1978; Anness *et al.*, 1979; Dickenson, 1979;

Wilson and Booer, 1979; Anness, 1980; 1981a; 1981b; Anness and Bamforth, 1982; Andrews, 1984). With the development of isolation and concentration techniques and the sensitivities of instruments, another lower concentration of sulphur compounds, DMDS, DMTS and dimethyl tetrasulphide (DMTeS), also have been investigated (Hashimoto and Kuroiwa, 1966; Kuroiwa and Hashimoto, 1970; Anderson and Howard, 1974; Peppard and Laws, 1979; Garza-Ulloa, 1980; Leppänen *et al.*, 1980; Arkima *et al.*, 1981; Williams and Gracey, 1982a; 1982b; Olsen *et al.*, 1988; Owades and Plam, 1988; Clarke *et al.*, 1991; Etiévant, 1991; Hardwick, 1995).

1.3.1 Flavour Threshold

DMS, DMDS, DMTS and DMTeS are pungent liquids with a strong "cooked vegetable" odour (cabbage, sweet corn, tomato and onion) which is objectionable in whisky. The flavour thresholds of these dimethyl polysulphides are shown in Table 1.2. Clearly, wide variations have been reported for the flavour thresholds of these sulphur compounds, perhaps due to variations of testing procedure, taste panels and samples. The threshold of DMTS is much lower than DMS and DMDS. The flavour thresholds of DMTS in malt and grain whisky new spirits, 15 and 4 ng/mL respectively, are higher than in other beverages possibly due to the masking effect of other flavours.

1.3.2 Foodstuffs and Beverages

DMS, DMDS and DMTS are detected in many foodstuffs and beverages (Tables 1.3 and 1.4). In *Allium* species, such as garlic and onion, and brassica vegetables, such as broccoli,

Table 1.2 Flavour threshold of sulphur compounds in beverages

Beverages	Flavour Threshold (ng/mL)				Ref
	DMS	DMDS	DMTS	DMTeS	
Water	10-12	0.6-30	0.005-3		a
Beer	25-50	6-10	0.01-0.2	0.2	b
White wine	22-25	29			c
Red wine	60				c
Ethanol 10%		20	0.1		d
Ethanol 23%			0.1		e
Malt whisky			15		f
Grain whisky			4		g

a. Schutte (1974), Buttery *et al.* (1976), Fors (1983), Wajon *et al.* (1985), Takeoka *et al.* (1991), Hansen *et al.* (1992)

b. Anderson and Howard (1974), Peppard (1978), Peppard and Laws (1979), Arkima *et al.* (1981), Stewart and Russell (1981), Williams and Gracey (1982a), Peppard (1985), Owades and Plam (1988)

c. Etiévant (1991)

d. Leppänen *et al.* (1979)

e. Perry (1989)

f. Beveridge (1990)

g. Whitby (1992)

Table 1.3 Food and food materials in which DMDS, DMTS and/or DMTeS were detected

Vegetables	Reference
Allium species	
Onion (cut, oil)	Carson and Wong[a,b] (1961), Brodnitz et al.[a,b] (1969), Boelens[a,b,c] (1971), Kallio and Salorinne[a,b] (1990), Kallio *et al.*[a,b] (1990), Kallio *et al.*[a,b,c] (1994)
Welsh onion	Kuo and Ho[a,b,c] (1992)
Chive	Kallio *et al.*[a] (1990)
Caucas	Nishimura *et al.*[a,b] (1971)
Garlic (cut, cooked)	Oaks *et al.*[a,b] (1964), Pino[d] (1992), Yu *et al.*[a,b] (1993), Ho *et al.*[a] (1995), Kim *et al.*[a] (1995)
Garlic (oil, extract)	Brodnitz *et al.*[b] (1971), Martin-Lagos *et al.*[a,b] (1995), Shaath *et al.*[a,b] (1995)
Brassica	
Broccoflower	Marks *et al.*[a,b] (1992)
Broccoli	Maruyama[b] (1970), Buttery *et al.*[a,b] (1976), Forney *et al.*[a] (1991), Hansen *et al.*[a] (1992), Marks *et al.*[a,b] (1992)
Cabbage	Bailey[a] (1961), MacLeod and MacLeod[a] (1970a,b), Buttery *et al.*[b] (1976), Marks *et al.*[b] (1992), Chin and Lindsay[b] (1993)
Cauliflower	MacLeod and MacLeod[a] (1970b), Buttery *et al.*[a,b] (1976), van Langenhove *et al.*[a,b] (1991), Marks *et al.*[a,b] (1992)
Brussels sprouts	MacLeod and MacLeod[a] (1970b), Maruyama[d] (1970), van Langenhove *et al.*[a,b,c] (1991), Marks *et al.*[a,b] (1992)

Table 1.3 (Continued)

Other vegetables & fruit	Reference
Mashroom	Borg-Karlson *et al.*[a,b] (1994)
Shiitake mashroom	Kameoka and Higuchi[b] (1976), Chen *et al.*[a,b] (1984), Chen *et al.*[c] (1986), Chen and Ho[a] (1986)
Tomato	Buttery[a] (1971)
Rutabaga	Hing and Weckel[a] (1964)
Strawberry	Ito *et al.*[a] (1990)
Potato (cooked)	Salinas *et al.*[a,b] (1994)
Pineapple	Takeoka *et al.*[b] (1991)
Wood garlic	Kubota and Kobatashi[a] (1994)
Meat	
Beef (cooked)	Hirai *et al.*[a] (1973), Garbusov *et al.*[a,b] (1976), Golovnya[a,b,c] (1980), MacLeod and Seyyedain-Ardebili[a] (1981), Golovnya[a] (1983), Bailey and Einig[a,b] (1989), Drumm and Spanier[b] (1991), Madruga and Mottram[a,b,c] (1995)
Beef (canned)	Persson and von Sydow[a,b] (1973)
Chicken (cooked)	Minor *et al.*[a] (1965), Nonaka *et al.*[a] (1967), Horvat[a] (1976)
Pork	Mussinan and Walradt[a] (1974), Withycombe *et al.*[b] (1976), Werkhoff *et al.*[a,b] (1993)
Lamb	Sutherland and Ames[a,b] (1995)
Fish & fish oil	Miller *et al.*[a,b] (1973), Christensen *et al.*[a,b,c] (1981), Josephson and Lindsay[a] (1986), Hsieh *et al.*[a,b] (1989), Cha and Cadwallader[a,b] (1995)

Table 1.3 (Continued)

	Reference
Cheese	McGugan *et al.*[a] (1968), Forss,[a] (1979), Parliment *et al.*[a,b] (1982), Lindsay and Rippe[a] (1986)
Milk (powder)	Hall *et al.*[a,b] (1985), Lee and Morr[a,b] (1994)
Egg	MacLeod and Cave[a,b] (1976)
Chocolate	Hoskin and Dimick[a] (1984a,b)
Cocoa	van Praag *et al.*[a,b] (1968), Baigrie[a,b] (1994)
Bread	Baltes and Song[a,b] (1994)
Potato chips	Deck *et al.*[a] (1973)
Nuts	Kinlin *et al.*[a] (1972)
Flour	Hwang *et al.*[a,b] (1994)
Soy sauce	Manley *et al.*[a] (1981)
Yeast extract	Ames and MacLeod[a,b] (1985), Werkhoff *et al.*[c] (1990), Ames and Elmore[a] (1992), Ames[a,b] (1994)

[a] DMDS was identified.

[b] DMTS was identified.

[c] DMTeS was identified.

Table 1.4 Beverages in which DMDS, DMTS and/or DMTeS were detected

Beverages	Reference
Beer	Anderson and Howard,[a] 1974), Garza-Ulloa[a] (1980), Leppänen *et al.*[a] (1980), Arkima *et al.*[a,b] (1981), Stewart and Russell[a,b] (1981), Williams and Gracey[a,b,c] (1982b), Seaton and Moir[a,b] (1987), Olsen *et al.*[a,b] (1988), Owades and Plam[a,b] (1988), Dercksen *et al.*[a,b] (1992)
Wine (red & white)	Leppänen *et al*[a]. (1979), Leppänen *et al.*[a] (1980)
Malt whisky spirits	Dellweg *et al.*[a] (1969), Masuda and Nishimura[a,b] (1981), Leppänen *et al.*[a,b] (1983), Beveridge[b] (1990)
Grain whisky spirits	Whitby[d] (1992)
Whisky	Dellweg *et al.*[a] (1969), Jounela-Eriksson[a,b] (1978), Leppänen *et al.*[a,b] (1979), Masuda and Nishimura[a] (1981), Leppänen *et al.*[b] (1983)
Brandy	Leppänen *et al.*[a,b] (1979)
Vodka	Leppänen *et al.*[a] (1979)
Rum	Leppänen *et al.*[a,b] (1979)
Awamori	Tamaki *et al.*[a,b] (1986)
Champagne	Maujean and Seguin[a] (1983a,b)
Potable water	Wajon *et al.*[b] 1985)
Milk	Shipe[a] (1980), Badings[a] (1991), Lee and Morr[a] (1994)

[a] DMDS was identified.

[b] DMTS was identified.

[c] DMTeS was identified.

cabbage, cauliflower and Brussels sprouts, DMDS and DMTS were detected before and after cooking. However, from meats, DMDS and DMTS were detected only after cooking. DMDS and DMTS were detected in many alcoholic beverages.

1.3.3 Changes of Sulphur Compounds during Fermentation

Leppänen *et al.* (1980) detected H_2S, methanethiol, methyl thioacetate, DMS and DMDS in synthetic medium of glucose, methionine and water, metabolised by *Saccharomyces cerevisiae*. Williams and Gracey (1982b) reported that both DMDS and DMTS decreased during beer fermentation presumably due to assimilation and metabolism by yeast. However, Beveridge (1990) reported an increased in DMTS during malt whisky fermentation. The differences of fermentation conditions, such as temperature, yeast strain, yeast type and hopping, may give these different results.

1.3.4 Change of Sulphur Compounds during Distillation

Several investigations of sulphur compounds during distillation have been published. Swan and Burtles (1978) have reported that DMS in the distillate from wash distillation in a copper pot still was reduced by 70% compared to all-glass distillation. Also, copper was involved in DMDS formation in wash distillation (Masuda and Nishimura, 1981). Although it was noted that the levels of DMDS and DMTS in the distillate increased with the duration and intensity of the heating (Leppänen *et al.*, 1983), the source could not be identified. The change of DMTS during both wash and spirit distillations in malt distilleries has been investigated (Beveridge, 1990). In wash distillation,

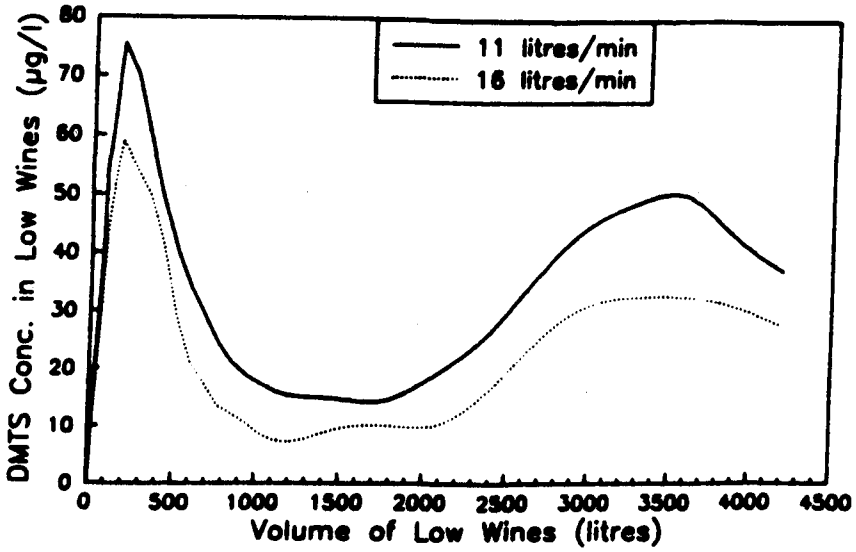

Figure 1.5 DMTS in low wines throughout wash distillation (Beveridge, 1990).

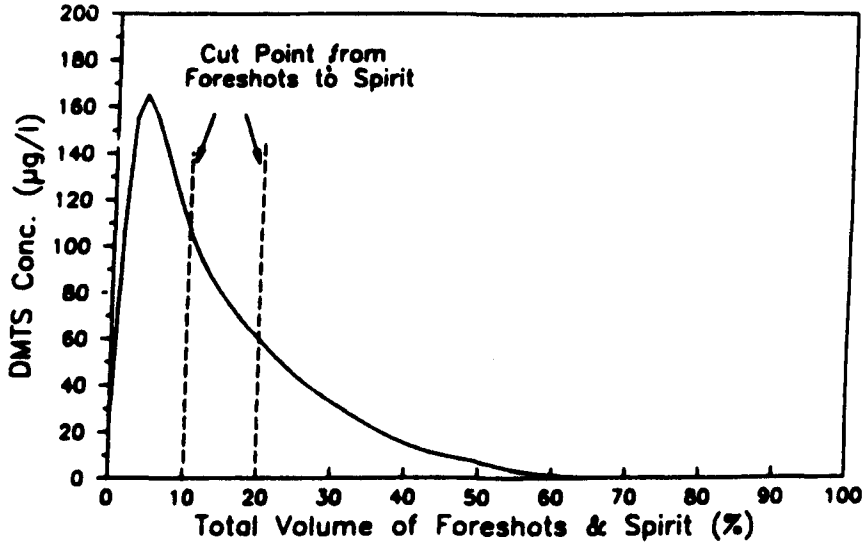

Figure 1.6 DMTS in foreshots and spirit during spirit distillation (Beveridge, 1990).

DMTS was highest at the start of distillation, and fell then increased with the distillation time (Figure 1.5). It is possible that the first peak shows the recovery of DMTS from wash and the second broad peak shows the formation of DMTS during wash distillation. In spirit distillation, DMTS was found largely in foreshots and early stages of the spirit fraction (Figure 1.6), however trace amounts were found in both feints and spent lees at 0.5 ng/mL. Therefore, a small amount of DMTS may occur in spirit distillation. The timing of switching from foreshots to spirit is critical in determining the DMTS level in final malt whisky spirit, as DMTS level in distillate decreases near the cut point. The recovery ratio of DMTS was only 40% in most cases but this ratio could vary between 10 and 70% (Beveridge, 1990).

Some malt whisky spirits, which contained over the flavour threshold level (15 ng/mL) of DMTS, were evaluated as 'heavy' (Duncan, 1988). It is doubtful that DMTS contributes solely to the 'heaviness' in malt whisky; it is possible that DMTS is one of several essential compounds in the 'heaviness' score. However, DMTS concentration level in grain whisky spirit is critical in whisky quality.

1.3.5 Reaction during Maturation

DMS, which contributes a substantial proportion of the immature malt whisky flavour, can disappear below the lower level of detection, 4 ng/mL, within one year in a charred cask, or within 3 years in a reused cask (Masuda and Nishimura, 1981; Perry, 1986; Philp, 1986). Table 1.5 shows the half lives of DMDS and DMTS in whisky spirits during maturation at four distinct alcohol strengths (Perry, 1986).

The level of DMTS declined more slowly than DMDS during maturation. These differences in half life were more pronounced at higher alcohol strength in malt whisky. As whisky spirits are filled into casks at 63-68% (v/v) alcohol strength in whisky industry, the decrease of DMTS is very slow. Therefore, it is very important to control the level of DMTS in malt whisky spirit. DMS, DMDS and DMTS levels decreased with an addition of gallic acid to malt whisky spirits during maturation experiments in glass (Philp, 1986).

Table 1.5 Reduction of polysulphides during maturation at a distinct alcohol strength (Perry, 1986)

Strength (% (v/v))	73	63	60	52
Half life of DMDS in Years	4.7	3.2	2.8	1.8
Half life of DMTS in Years	28	38	19	5.8

1.4 Model Reaction System

1.4.1 Maillard Reaction

It is well known that many flavour components are formed during food processing, especially cooking. During the thermal processing of foods and beverages, the non-enzymatic browning reaction between the amino group of amino acids, peptides, or proteins and the carbonyl group reducing sugars, takes place. Since this well-known reaction was reported by Maillard in 1912 (Mottram, 1994), many studies

based on the Maillard reaction have been performed by food and flavour chemists.

1.4.2 Model System of DMDS Formation

Ballance (1961) reported trace amount of DMDS was identified after heating DL-methionine with ninhydrin at 80 °C for 2 h. Casey *et al.* (1963) identified DMDS from another methionine model system with pectin, 100 °C, pH 6.5. This reaction is well known as Strecker degradation of amino acid with diketone. Arroyo and Lillard (1970) also identified DMS and DMDS from methionine model system with glucose at 98±2 °C, pH 6.5-8.0. Microbial formation of DMDS from methionine has been reported (Kadota and Ishida, 1972).

DMTS formation from methionine was suggested by Griffith and Hammond (1989), but without experimental detail. The experimental conditions which have been published to date are summarised in Table 1.6. None have simulated the conditions, pH 3-5, temperature up to 100 °C, of malt whisky distillation.

Generally, the quantity of sulphur containing compounds increased with an increase of pH (Chan and Reineccius, 1994). Oxygen was suggested to participate in the formation of DMDS from methanethiol (Schutte, 1974). Also a strong oxidant, such as H_2O_2, or ascorbate plus Cu catalyst increased the formation of DMDS and DMTS from methanethiol and H_2S (Chin & Lindsay, 1994a) and from methionine (Lieberman *et al*, 1965). Carbonyl compounds, such as aldehydes, ketones and reducing sugars, are involved the degradation of methionine (MacLeod, 1994).

Table 1.6 Model systems in which DMDS and other sulphur compounds were identified

Model System [a]	Other	pH	Temp (°C)	Time (h)	Reference
Methionine, methional/sulphite [b,g]	-	3-5	24	2	Wainwright (1972)
Methionine/methylglyoxal [d,f]	-	-	25	-	Griffith and Hammond (1989)
Methionine/ascorbate [c,g]	Cu^{2+}	6.8	30	0-1.7	Lieberman *et al.* (1965)
Methanethiol/H_2S/ascorbate [d]	Cu^{2+},Fe^{3+},PO_4^{2-}	6.3	30	5	Chin and Lindsay (1994a)
Methyl methanethiosulphinate/H_2S [c,d]	PO_4^{2-}	6.3	30	0-20	Chin and Lindsay (1994b)
Methionine/ninhydrine [b,c]	PO_4^{2-}	-	80	2	Ballance (1961)
Methionine/pectine [b,c]	PO_4^{2-}	6.5	100	-	Casey (1963)
Methionine/glucose [b,c]	(HCl)	6.5-8.0	98±2	2	Arroyo and Lillard (1970)
Methionine, S-methylmethionine [h]	O_2/N_2	-	100	2	Miglio (1971)
HVP/cysteine/xylose [d,f]	Fe^{2+},Mn^{2+},Fe^{3+}	-	100	4	Mussinan and Katz (1973)
Methionine/glucose [f]	PO_4^{2-}	6-8	75-115	0.5-7.5	Chan and Reineccius (1994)
Thiamine	-	1.5-9.5	130	2-6	Güntert *et al.* (1992)
Methionine/thiamine [d,e,f]	-	3.3	130	6	Güntert *et al.* (1993)
Thiamine	-	2.3	135	0.5	Hartman *et al.* (1984)
Methionine/ribose [d,f]	$P_2O_7^{4-}$	4.5-6.5	140	1	Mottram and Leseigneur (1990)
Methionine/ribose [d,f]	$P_2O_7^{4-}$	4.5-6.5	140	1	Meynier and Mottram (1995)
Casein hydrolysate/glucose [d]	-	7.5	180	1	Zhang *et al.* (1992)
Methionine,methioninesulphoxide/glucose [f,e]	-	-	180	1	Yu and Ho (1995)
Cysteine/methionine/furfural	-	-	200-220	1	Silwar and Tressl (1989)

[a] DMDS was identified in all model systems. [b] DMS was also identified. [c] CH_3SH was also identified.
[d] DMTS was also identified. [e] DMTeS was also identified. [f] Methional was also identified.
[g] Methionine sulphoxide/sulphone was also identified. [h] Diethylsulphide was also identified.

1.5 Objectives

There are two main reasons why it is very important to control the level of sulphur compounds, especially DMTS, in whisky spirits. Firstly, sulphur compounds have very low flavour thresholds. DMTS has a much lower threshold than DMS and DMDS and, although DMTS gives whisky a desirable "heaviness", too high level is considered objectionable. Secondly, the level of DMTS in whisky declines more slowly than both DMS and DMDS during maturation.

The information on mechanisms of formation of DMDS and DMTS during distillation is limited. The aim of this project was therefore to investigate the formation of DMDS and DMTS and the factors which affect DMDS and DMTS formation from precursors during distillation in order to control the level of these sulphur compounds in whisky spirits.

These sulphur compounds already exist in the fermented wash. This project is focused on the formation and reactions of these sulphur compounds during distillation. A better understanding of these factors should allow the control and optimisation of the level of sulphur compounds in whisky spirits.

Methional and methionine were used for the precursors of DMDS and DMTS. The following factors that may affect the formation of DMDS and DMTS from precursors were evaluated: heating time, pH of the wash (reation mixtures), oxygen (aerobic and anaerobic conditions or oxidant), and catalysts, especially copper salts.

2. MATERIALS AND METHODS

2.1 Determination of Concentration of Sulphur Compound

2.1.1 Reference Chemicals

The volatile sulphur compounds for preparing calibration curves were obtained commercially as follows:

DMS	Aldrich, Gillingham, UK
DMDS	Sigma, Poole, UK
DMTS	Fujimoto Chemical, Tokyo, Japan
Methional [3(methylthio)propionaldehyde]	Aldrich
Ethyl methyl sulphide (EMS) (internal standard)	Aldrich
Di-n-decyl sulphide (internal standard)	Tokyo Chemical Industry, Tokyo, Japan

2.1.2 Direct Injection Method

This method was used for the analysis of DMS, DMDS and methional in the samples.

2.1.2.1 Direct Injection Method for Analysis of Samples from the Thermal Reaction of Methional

An internal standard solution (50 μL) consisting of 20 μg/mL EMS in 60% (v/v) ethanol solution was added to a thermally reacted sample (1.0 mL). The sample and internal standard solution were cooled by submerging in crushed ice before mixing. After mixing for 10 s, a 3 μL portion of the

mixture was analysed by gas chromatograph equipped with a flame photometric detector.

2.1.2.2 Direct Injection Method for Analysis of Samples from the Thermal Reaction of Methionine

The thermally reacted sample (1.0 mL) was mixed with 100 μL of an internal standard solution consisting of 550 μg/mL di-n-decyl sulphide in 60% (v/v) ethanol solution. The sample was iced before mixing. After mixing for 10 s, a 3 μL portion of the mixture was analysed by gas chromatography.

2.1.3 Solvent Extraction Method

This method was used for the quantitative analysis of DMTS in the samples. HPLC grade pentane (Rathburn chemicals, Walkerburn, UK) and anhydrous dichloromethane (Aldrich) were carefully purified by redistilling individually on a Schneider column (Kikotech, Osaka, Japan).

This extraction method can only be used for volatile lipophilic volatile compounds. Some hydrophilic compounds may be removed from the eluate.

2.1.3.1 Column Packing Procedure

Extrelut®20 (Merck, Lutterworth, UK) was used for removing ethanol and water from the column eluate. The 10 mm round filter was set on the sintered glass filter in a glass column (24 cm, 3 cm i.d.). The contents of one sachet of Extrelut®20 were poured into the glass column. For the material to settle and pack evenly, the glass column was

vibrated for 10-20 s. Finally, a 24 mm round filter was set on top of the column packing material in the glass column.

2.1.3.2 Sample Preparation

A 20 mL sample, in approximately 60% (v/v) ethanol solution, was mixed with 50 μL of an internal standard solution, consisting of 20 μg/mL di-n-decyl sulphide in 60% (v/v) ethanol, in a 100 mL beaker. The mixture was transferred to the top of the column, and allowed to soak into the Etrelute®20 for 15 min. A needle was attached to the bottom of the column. The column was filled with 60 mL 10:1 (v/v) of pentane and dichloromethane. The eluate was concentrated to 0.1-0.5 mL in a Kuderna-Danish evaporative concentrator (Kikotech, Osaka, Japan) with a Schneider column (Heath and Reineccius, 1986) in water bath at 45 °C. A 3 μL portion of the extract was then analysed by gas chromatography.

2.1.4 Gas Chromatographic Analysis

A Hewlett Packard 5890 Series II gas chromatograph (Stockport, UK) equipped with a flame photometric detector (FPD, HP 19256A) was used to analyse the sulphur compounds. The FPD outputs were monitored using an HP Vectra QS/165 with ChemStation (ver.A.02.12) chromatographic software , or an ELONEX PC450/VL with ChemStation (ver.A.03.34) chromatographic software. For both the direct injection method and the solvent extraction method, the analytical column and operating conditions were as follows:

Column: J & W Scientific (Folsom, CA, USA) DB-WAX megabore fused silica capillary column (30 m, 0.53 mm i.d.) coated with 1.0 μm polyethylene glycol film.

Gas flow rate

Carrier gas (He):	110 mL/min (split ratio, 2:9)
Septum purge gas (He):	5 mL/min
Makeup gas (N_2):	25 mL/min
H_2 gas:	110 mL/min
Air:	85 mL/min.

Split/Splitless Injection program: Splitless mode for 1 min from the injection, then split mode.

Injector temperature: 250 °C

Detector temperature: 250 °C

Temperature program: 40 °C (0 min), 40-220 °C (20 °C/min), 220 °C (6 min)

2.1.5 Identification

Sulphur compounds were confirmed by co-injection of authentic compounds with samples using two different polarity columns (J&W Scientific DB-WAX megabore fused silica capillary, 30 m, 0.53 mm i.d., coated with 1.0 μm polyethylene glycol film, and Hewlett Packard Ultra 2 fused silica capillary, 50 m, 0.32 mm i.d., crosslinked with 0.52 μm 5% phenyl methyl silicone)

2.1.6 Calibration

Calibration curves were obtained with 60% (v/v) alcoholic solution containing 50-1000 ng/mL DMS and DMDS and 1-50 mg/mL methional for the direct injection method, and containing 0.1-50 ng/mL DMTS for extraction method.

2.2 Colorimetric Determination of Cupric ion in Reaction Mixtures

The method was that of Middleton (1965). The volume of sample was changed one tenth.

2.2.1 Chemicals

The following chemical compounds were used for this method.

Neutral red	Sigma, Poole, UK
Sodium citrate	Sigma
Sodium hydroxide (pellets)	BDH, Lutterworth, UK
Ammonium acetate	BDH
Ammonium hydroxide	Sigma
Acetic acid	BDH
Bis-cyclohexanone oxaldihydrazone	Sigma
Copper atomic absorption standard solution (1.005 mg/mL of copper in 1% (w/w) HNO_3)	Aldrich

Water was purified with a Millipore Milli-Q Organex water system.

Aqua regia ($HCl:HNO_3:H_2O$ = 2:1:1 (v/v)) was used to remove all metals from all apparatus.

2.2.2 Reagents

The following reagents were prepared for this method.

Neutral red indicator: 0.003% (w/v) aqueous neutral red.

Alkaline citrate reagent: 2N sodium citrate solution in 2N sodium hydroxide.

Ammonium acetate reagent:1.6 N ammonium acetate solution was adjusted to pH 9.1 by adding 0.4 N ammonium hydroxide solution.

Bis-cyclohexanone oxaldihydrazone reagent:0.5% (w/v) in 50% (v/v) ethanol solution.

2.2.3 Procedure

The sample solution was filtered through a 0.45 μm membrane filter (Ekicrodisc 25, Gelman Sciences, Tokyo, Japan) to remove suspended matter. A volume (max. 3.5 mL) of the sample was taken to give an absorbance below 1.0. The sample and 0.1 mL neutral red indicator reagent were added to a 5 mL volumetric flask with an octagonal stir bar, 1.3 X 3.2 mm, (Sigma-Aldrich Techware, Poole, UK). The solution was neutralised by alkaline citrate reagent, and 0.5 mL of ammonium acetate reagent was added. After the mixture was cooled in the refrigerator for 10 min, 0.1 mL bis-cyclohexane oxaldihydrazone reagent was added. The solution was made up to 5 mL, and was stirred well. After 30 min, the optical density of the solution was measured at 600nm in a 1 cm cell, using a spectrophotometer (Shimadzu, UV-240, Kyoto, Japan).

2.2.4 Calibration

A calibration graph was obtained with 7% (v/v) ethanol solution containing 0.1-5.0 μg/mL cupric ion using Aldrich copper atomic absorption standard solution (Figure 2.1).

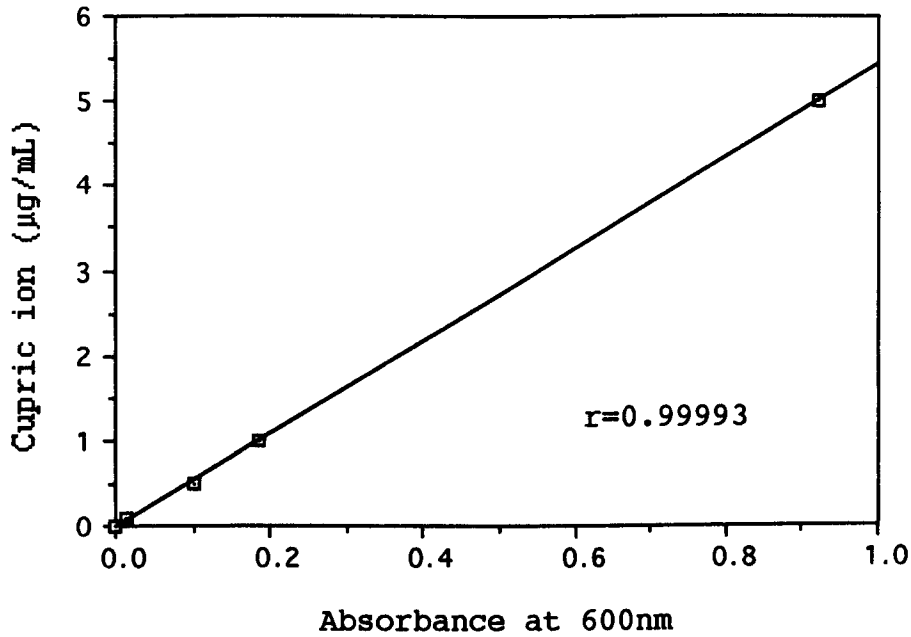

Figure 2.1 Calibration graph of cupric ion determination by colorimetric method

2.3 Thermal Reactions of Sulphur Compounds

2.3.1 Chemicals

The following compounds were used for the thermal reaction of L-methionine and methional.

L-methionine	Sigma
Methional	Aldrich
Sodium phosphate	Sigma
Sodium sulphate anhydrous	BDH
L-Lactic acid (85 % in water)	Aldrich
Nitrogen gas (99.97%)	BOC Ltd., London, UK
Oxygen gas (99.995%)	BOC

2.3.2 Reaction Mixtures for Thermal Reaction of Methional or Methionine without Buffer

When the effect of copper salt on the formation of sulphur compounds was evaluated, 157 μM copper salt, equivalent to 10 μg/mL Cu(II), was added to 360 mL of 7% (v/v) ethanol solution containing 96 μM methional or 100 μM L-methionine. The reaction mixture was not buffered and the pH was not adjusted during experiment.

2.3.3 Reaction Mixtures for Thermal Reaction of Methional or L-Methionine with Phosphate or Sulphate

Anhydrous disodium hydrogen phosphate (Sigma), 255.5 mg, equivalent to 474.9 μg/mL (5 mM) PO_4^{2-}, or anhydrous sodium sulphate (BDH), 102.3 mg, equivalent to 192.1 μg/mL (2 mM) SO_4^{2-}, was added as a buffer to 360 mL ethanol solution, 7% (v/v), containing 96 μM methional or 100μM L-methionine. For addition of cupric ion, 157 μM copper salt, equivalent to

10 μg/mL Cu(II), was added to the reaction mixtures. The pH of solutions were adjusted to 3.0, 4.0, 5.0, 6.0, 7.0 or 8.0 by the addition of L-lactic acid (Aldrich) or sodium hydroxide (BDH) solution.

2.3.4 Model Distillation System for Thermal Reaction

A reaction mixture, 360 mL 7% (v/v) ethanol solution, was distilled in duplicate in the apparatus designed by Saita *et al.* (1986), for 30 min except the experiment of the effect of duration of distillation, and 120 mL of distillate was collected in a 250 mL measuring cylinder. The distillation apparatus was composed of 500mL Erlenmeyer flask, a 2000 mL foam holding space, a distillation head and condenser (Figure 2.2). Three porous ceramic cubes (Schmacker, Germany) were used for boiling stones. A Bunsen burner was used as heating source with a ceramic laboratory plate (BDH) and a tripodal stand.

When aerobic and anaerobic conditions were evaluated, either O_2 or N_2 gas was bubbled into the reaction mixture through a glass tube with sintered glass, which was fitted to the side of an 500 mL Erlenmeyer flask, to create aerobic or anaerobic conditions. Both gas flow rates were 50 mL/min

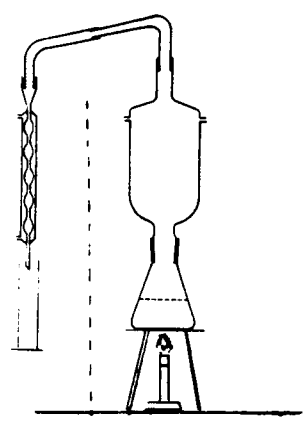

Figure 2.2 The model distillation apparatus

during distillation. Aluminum fence was put between the distillation unit and a cylinder to aviod a fire risk.

2.3.5 Reaction Mixtures for Thermal Reaction of Methional or L-Methionine with Phosphate Buffer

An equimolar amount (1.0 mM) of L-methionine and reducing sugar were added to 11 mL of 7% (v/v) ethanol solution, containing 5 mM anhydrous disodium hydrogen phosphate in a 12 mL centrifuge tube with a screw cap (12 mL, Iwaki Glass, Tokyo, Japan, Cat. No.6125). When the effect of copper salt on the formation of sulphur compounds from L-methionine was evaluated, 157 μM $CuAc_2$ was added to the reaction mixture. The pH of the reaction mixtures were adjusted to 4.5 by the addition of L-lactic acid.

2.3.6 Sealed Vial Thermal Reaction System

Before thermal reaction, either O_2 or N_2 gas was passed into the reaction mixture at 50 mL/min for 15 min through a sintered glass bubble to create aerobic or anaerobic conditions respectively. Before the screw cap was closed, the gas in the headspace was replaced by either O_2 or N_2 gas. as approximate. The reaction mixture was heated for 3 h at 100 °C in the water bath (Büchi 461 water bath, Flawil, Switzerland). After reaction, the centrifuge tube was cooled down quickly by ice.

The volume of sample must not be exceeded to avoid the explosion of a sealed vial.

3. RESULTS

3.1 Optimisation of Determination of Sulphur Compounds

To determine the concentration of sulphur compounds, such as DMS, DMDS and DMTS, in the samples, several trials were performed on the optimisation of analytical methods by the instruments available in this department.

3.1.1 Development of Direct Injection Determination of Sulphur Compounds

3.1.1.1 Determination of Sulphur Compounds Using the Packard Model 428 Gas Chromatograph

The first trials for the determination of sulphur compounds were performed using a Packard Model 482 gas chromatograph equipped with a Flame Photometric Detector (FPD), fitted with a capillary column (J&W, GS-Q, 30 m, 0.53 mm i.d.) and connected to A SP4270 chromatography integrator (Spectra-Physics). The method described in section 2.1.2 was used for the direct injection analysis.

The flow rates of gases were adjusted for maximum FPD sensitivity. The optimum operating conditions of this gas chromatograph were as follows:

Carrier gas (N_2):	15 mL/min (splitless mode)
Hydrogen gas:	150 mL/min
Air:	55 mL/min
Oven temperature:	100 °C
Injection temperature:	150 °C
Detector temperature:	150 °C

Over 4 μg/mL DMS could be detected under these conditions (Figure 3.1). According to technical information provided by J&W, a GS-Q column does not have a liquid stationary phase, but a solid, porous divinylbenzene homoplymer. Therefore, the next trial employed a high performance fused silica capillary column coated with polyethylene glycol (J&W, DB-WAX, 60 m, 0.25 mm i.d., 0.25 μm film thickness). The flow rate of carrier gas was 1.7 mL/min. The oven temperature of the gas chromatograph was changed from 100 °C to 90 °C to separate the peaks of DMS and EMS internal standard from ethanol. DMS and DMDS could be detected at concentration of 40 ng/mL (Figure 3.2). The reproducibility of DMS and DMDS measurements was evaluated using a standard solution (40 ng/mL DMS and 40 ng/mL DMDS) with an internal standard, the coefficients of variation of those compounds were 7.1% and 8.0% respectively due to baseline noise (Table 3.1).

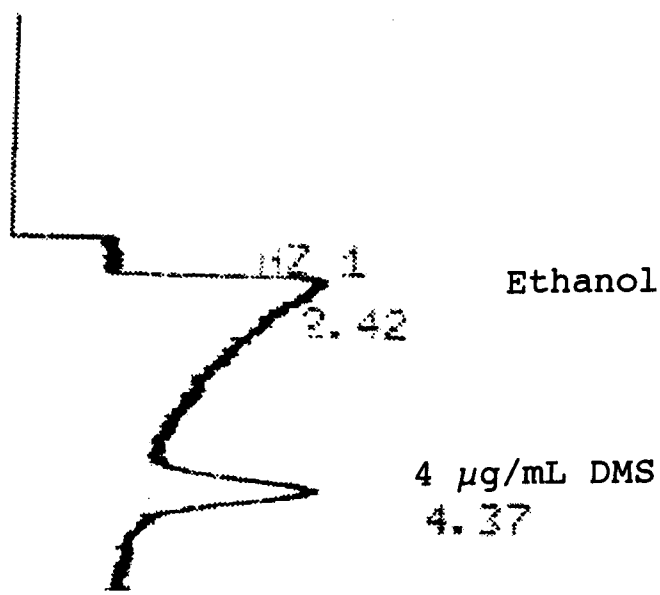

Figure 3.1 Gas chromatogram of 4 μg/mL DMS standard solution using Packard Model 428 GC with a GS-Q column

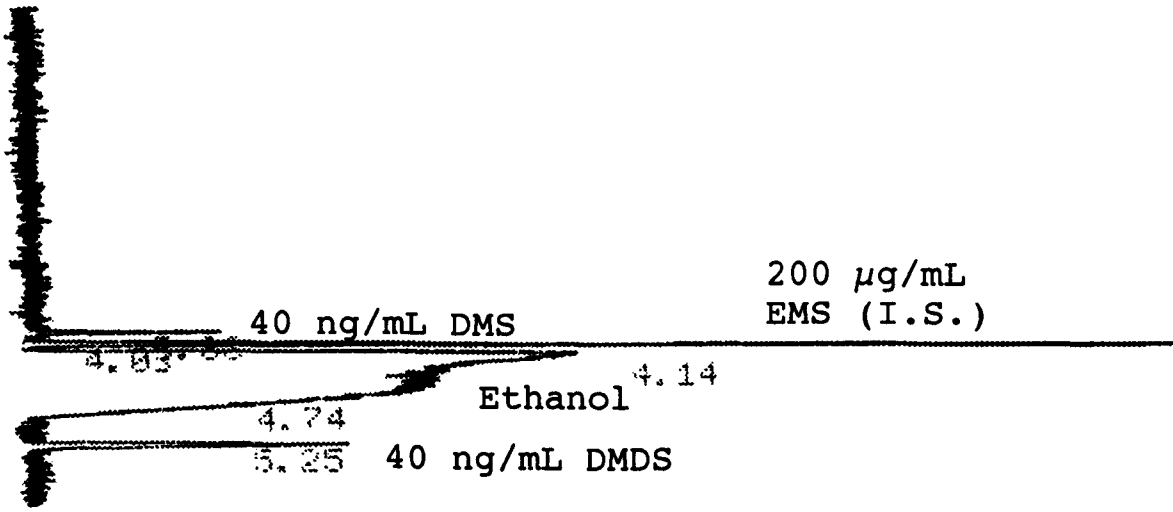

Figure 3.2 Gas chromatogram of 40 ng/mL DMS and DMDS standard solution using Packard model 428 GC with a DB-WAX (60 m, 0.25 mm i.d.) column

Table 3.1 Reproducibility of sulphur compounds analysed using a Packard Model 428 gas chromatograph equipped with a FPD

Run No.	DMS (ng/mL)	DMDS (ng/mL)
1	45.22	36.89
2	43.09	41.30
3	40.40	36.14
4	42.21	44.36
5	36.14	40.73
6	43.68	70.03
7	43.91	61.49
Mean	41.97	39.54
SD	2.96	3.17
CV (%)	7.1	8.0

It was difficult to achieve a high sensitivity using a Packard model 482 gas chromatograph equipped with a FPD. Some days it was impossible to adjust this machine to acceptable sensitivity.

3.1.1.2 Trial of Determination of Sulphur Compounds Using Chrompack CP9000 Gas Chromatograph

The next trial was performed using a Chrompack CP9000 gas chromatograph equipped with Sievers Sulphur Chemiluminesence Detector (SCD) 350B and connected to a Chrompack Integrator System (IBM personal computer System2, Model 30 286 with Mosaic chromatographic software). Also, to improve the response to each sulphur compound, two different fused silica capillary column coated with polyethylene glycol (J&W, DB-WAX) were investigated. A comparison of two gas chromatograms of 100 ng/mL DMS by 0.25 and 0.53 mm i.d. columns is shown in Figures 3.3 and 3.4. Although the sensitivity of the analysis by the widebore column was better than the narrow column, there were too many noise signals on the baseline. These spike noises were disappeared when a SP4270 chromatography integrator (Spectra-Physics) was used (Figure 3.5). Therefore, they were probably caused by the integrator computer.

The optimum operating conditions of the gas chromatograph were as follows:

Gas flow rate

Carrier gas(He):	110 mL/min	(split ratio, 2:9)
Makeup gas (N_2):	30 mL/min	
H_2 gas:	30 mL/min	
Air:	250 mL/min	

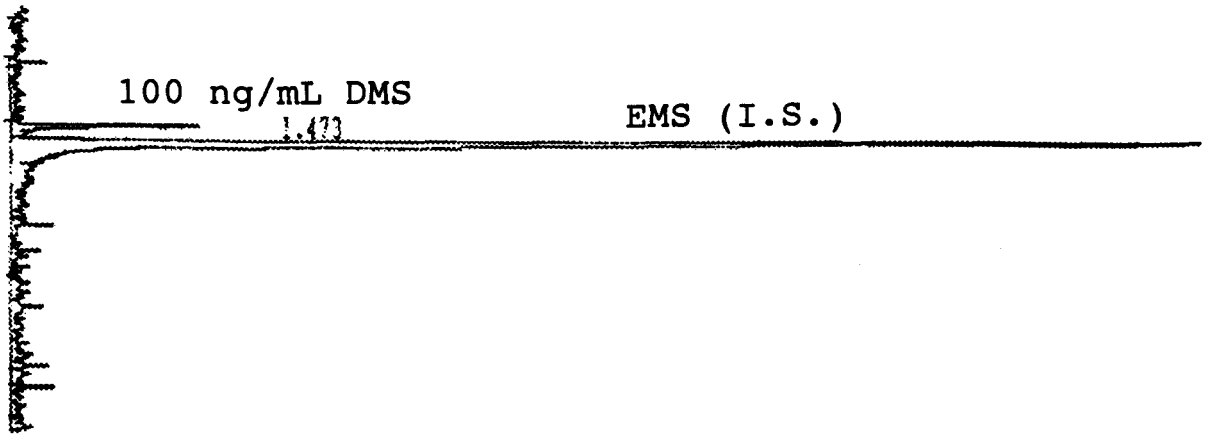

Figure 3.3 Gas chromatogram of 100 ng/mL DMS using Chrompack CP9000 GC with DB-WAX (60 m, 0.25 mm i.d.)

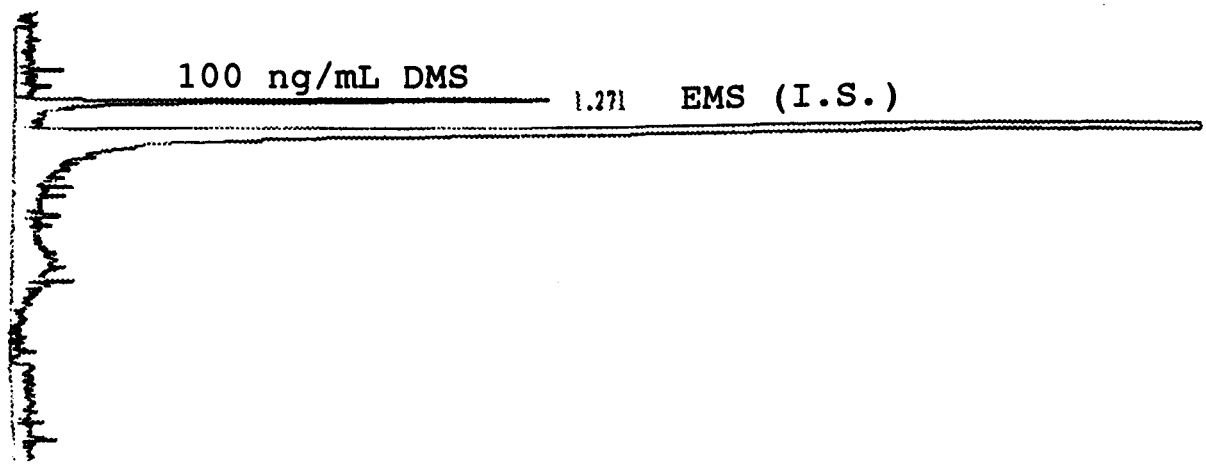

Figure 3.4 Gas chromatogram of 100 ng/mL DMS using Chrompack CP9000 GC with DB-WAX (30 m, 0.53 mm i.d.)

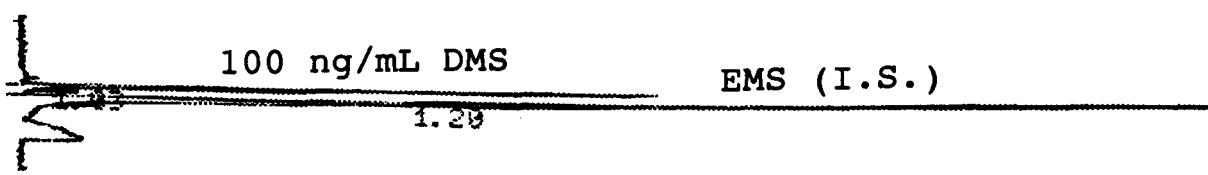

Figure 3.5 Gas chromatogram of 100 ng/mL DMS using Chrompack CP9000 GC with DB-WAX (30 m, 0.53 mm i.d.) and SP4270 integrator

Oven temperature:	100 °C
Injection temperature:	200 °C
Detector temperature:	225 °C
Temperature program:	70 °C (4 min), 70-170 °C (10 °C/min), 170 °C (30 min)

The reproducibility of DMS and DMDS measurements was checked with a standard solution (20 ng/mL of DMS and 100ng/mL of DMDS) with an internal standard, the coefficients of variation of those compounds are shown in Table 3.2.

Table 3.2 Reproducibility of sulphur compounds analysed using a Chrompack CP9000 gas chromatograph equipped with a Sievers SCD 350B (n=5)

Standard sample	Coefficient of variation (%)
20 ng/mL DMS	9.4
100 ng/mL DMS	2.2
20 ng/mL DMDS	7.6
100 ng/mL DMDS	2.3
100 ng/mL DMTS	7.6

The coefficient of variation using capillary column gas chromatography is commonly less than 10% (Hoyt and Smith, 1991). Therefore, this result was satisfactory, However, it is not possible to use the Chrompack CP9000 gas chromatograph, the same trial was performed using the

Hewlett Packard Gas Chromatograph Model 5890 Series II equipped with FPD.

3.1.1.3 Trial of Direct Injection Determination of Sulphur Compounds Using Hewlett Packard Gas Chromatograph Model 5890 Series II Equipped with Flame Photometric Detector

The capillary column DB-WAX (30 m, 0.53 mm i.d.) was used, and each gas flow rate was adjusted to the rate recommended by Hewlett Packard. The oven temperature was adjusted to achieve the highest peak, good separation and quick analysis. The optimum conditions were described in section 2.1.4. A typical gas chromatogram of 100 ng/mL DMS and DMDS is shown in Figure 3.6. Under the these conditions, the good reproducibility were obtained as with the Chrompack CP9000 system (Table 3.3).

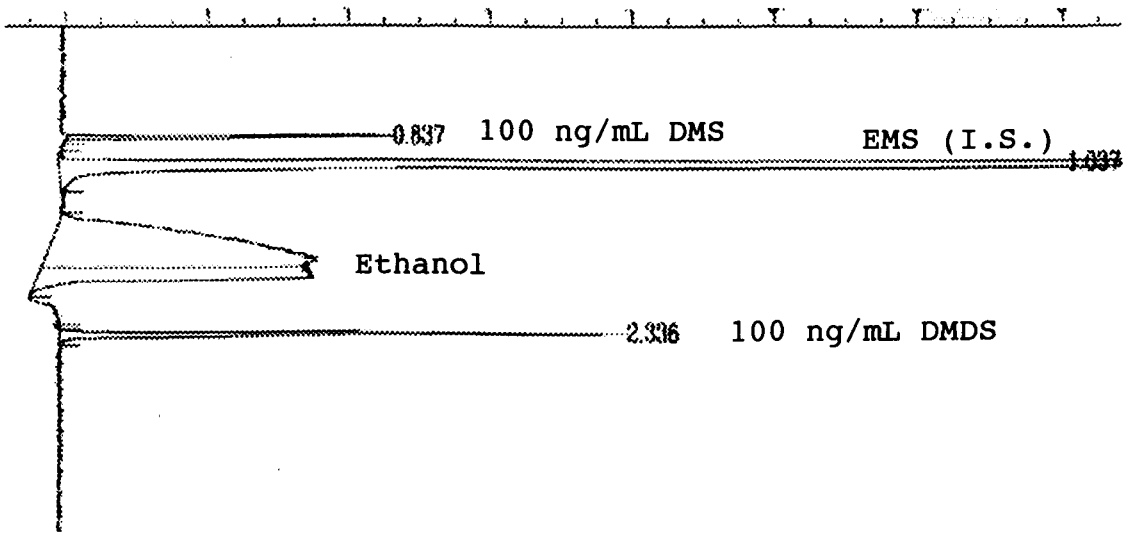

Figure 3.6 Gas chromatogram of 100ng/mL DMS and DMDS using HP gas chromatograph model 5890 series II with FPD

This result was satisfactory, as the coefficient of variation using capillary column gas chromatography is commonly less than 10% (Hoyt and Smith, 1991).

Table 3.3 Reproducibility of sulphur compounds analysed by the Hewlett Packard gas chromatograph Model 5890 Series II equipped with a FPD

Run No.	DMS (ng/mL)	DMDS (ng/mL)
1	49.62	51.00
2	50.00	48.08
3	52.28	49.52
4	49.72	50.21
5	52.41	50.75
Mean	50.81	49.91
SD	1.26	1.05
CV (%)	2.5	2.1

3.1.1.4 Calculation Method for Calibration Graphs of Sulphur Compounds Analysis by FPD-GC

The calibration graphs were obtained with a 60% (v/v) ethanol solution containing 50-1000 ng/mL DMS and DMDS and 1-50 mg/mL methional by the direct injection method described in section 2.1.2. It is well-known that the FPD has a nonlinear response to sulphur compounds (Hysert *et al.*, 1979; Marriott and Cardwell, 1981; Williams and Gracey, 1982a; Lee and Siebert, 1986). There are two calculation techniques to solve this problem. The log-log method (Lee

and Siebert, 1986) and the square root method (Hysert *et al.*, 1979; Williams and Gracey, 1982a). The calibration graphs of DMS and DMDS using these two methods are shown in Figures 3.7, 3.8, 3.9 and 3.10. Although the correlation coefficients of these graphs were above 0.9995, further improvement of the square root method was evaluated. Figures 3.11 and 3.12 show the DMS and DMDS calibration using formula.

$$\text{Response} = (\text{peak area}/\text{internal standard area}) \times (\text{internal standard height}/\text{peak height})^{1/2}$$

The correlation coefficients of DMS and DMDS from these calibration graphs were 0.99988 and 0.99980 respectively. This calculation method which gave the highest linearity of graphs was used for the measurement of sulphur compound concentration throughout this project.

The calibration graph for methional was obtained using the same method. As a ghost peak overlapped on the EMS (internal standard) peak, 550 μg/mL di-n-decyl sulphide in 60% (v/v) ethanol solution was used as an internal standard.

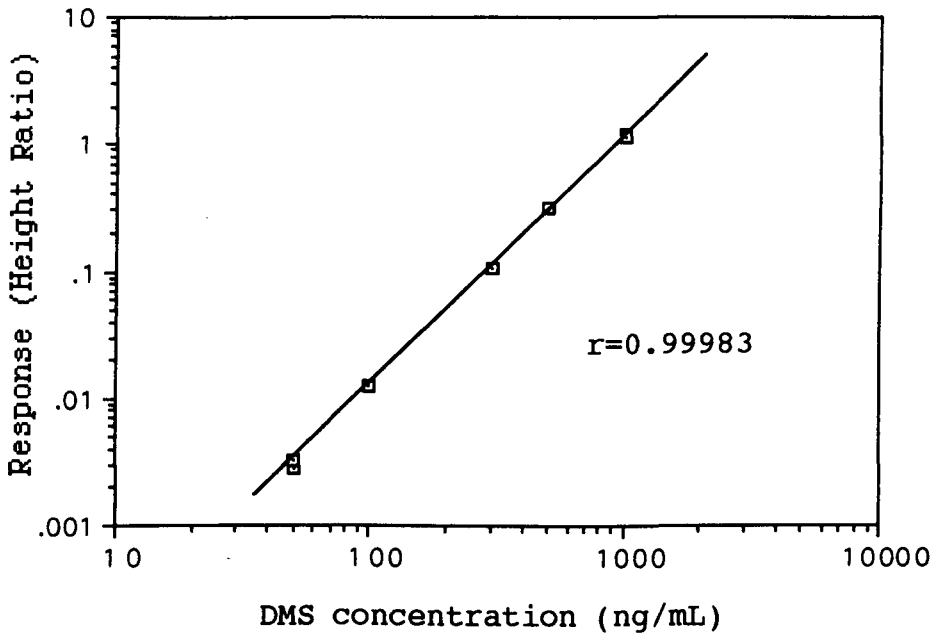

Figure 3.7 Calibration of DMS using a log-log scale

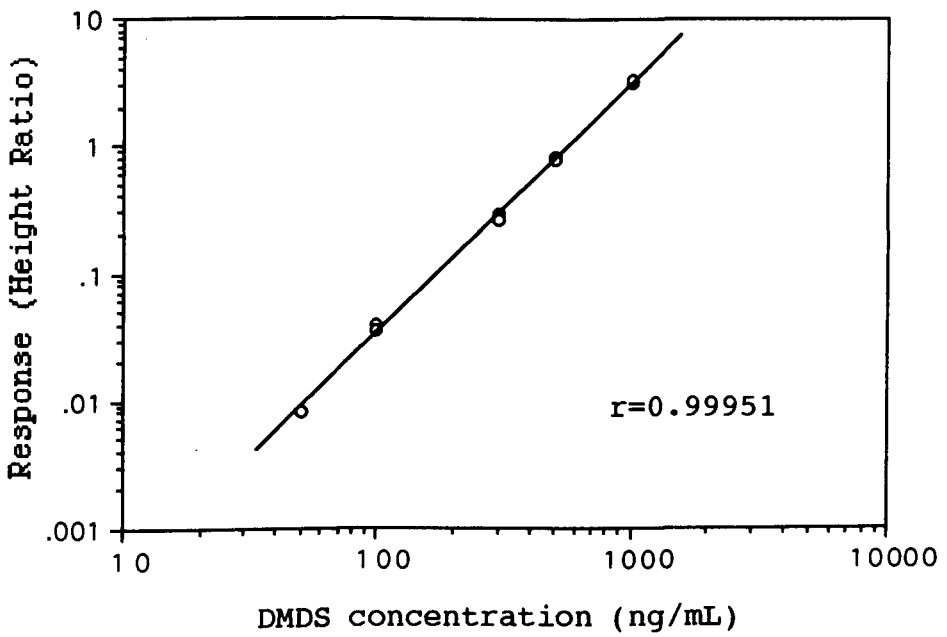

Figure 3.8 Calibration of DMDS using a log-log scale

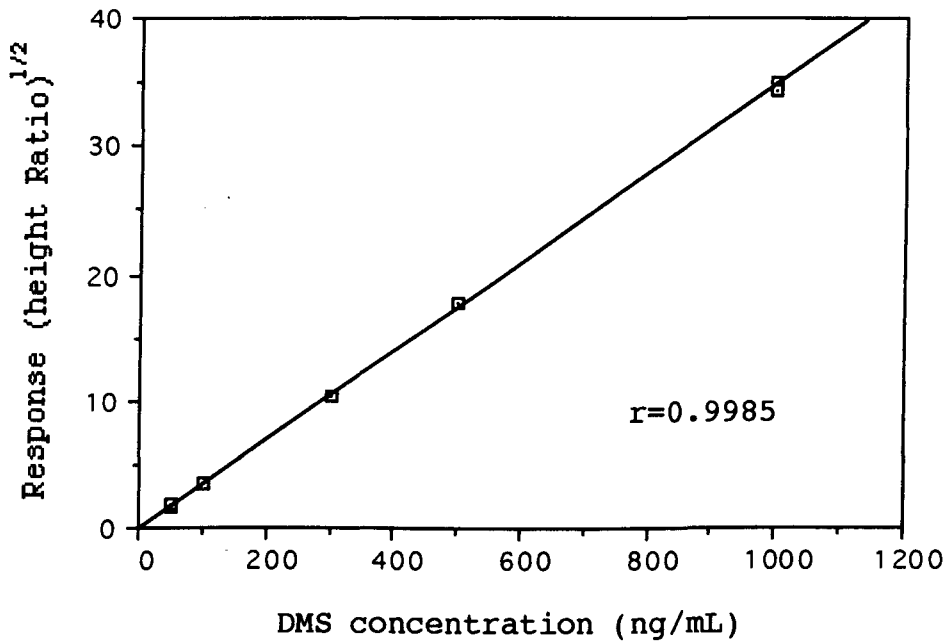

Figure 3.9 Calibration of DMS

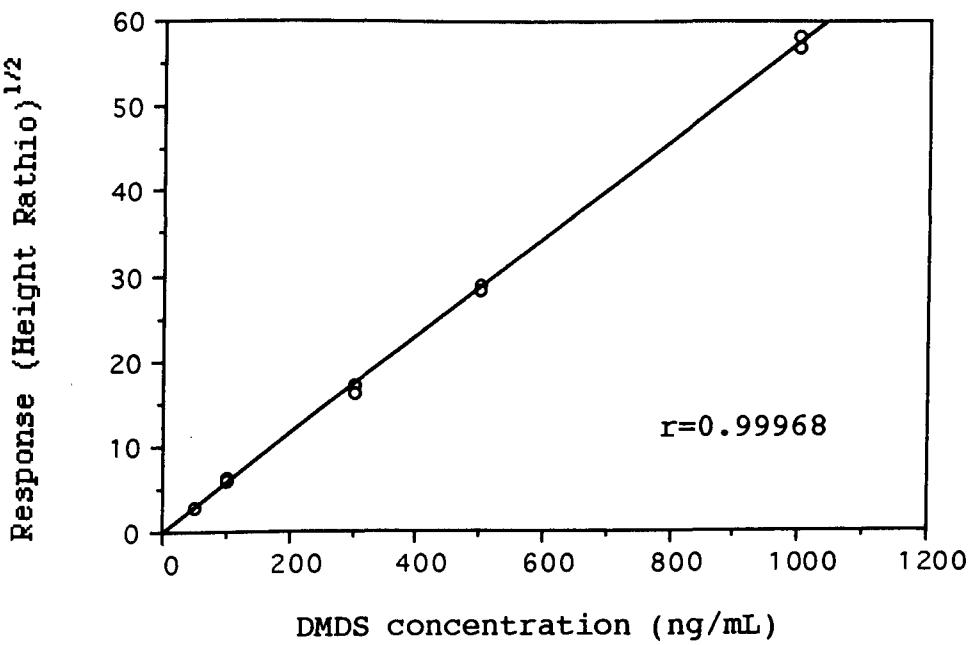

Figure 3.10 Calibration of DMDS

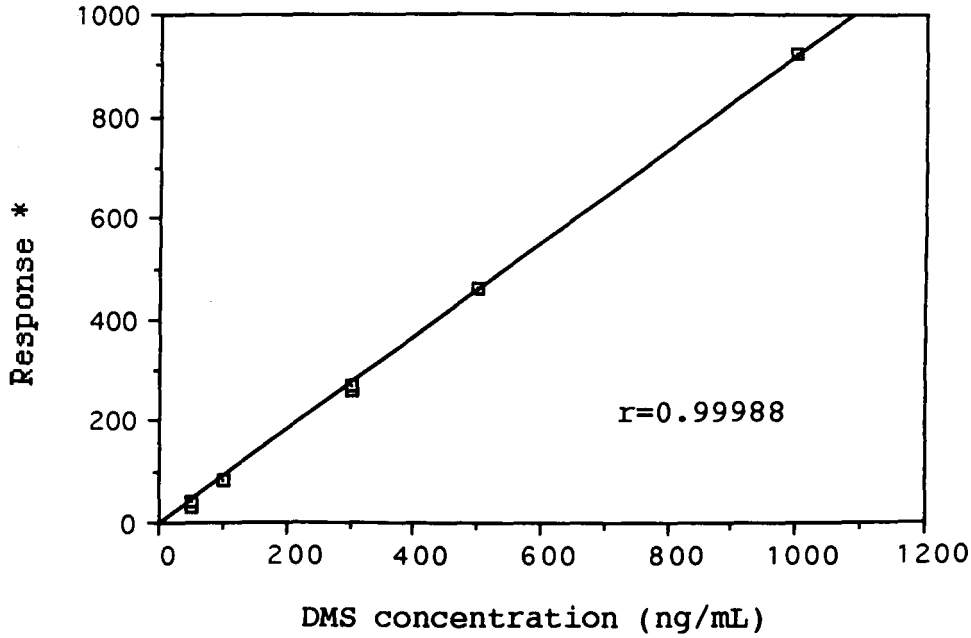

Figure 3.11 Calibration of DMS

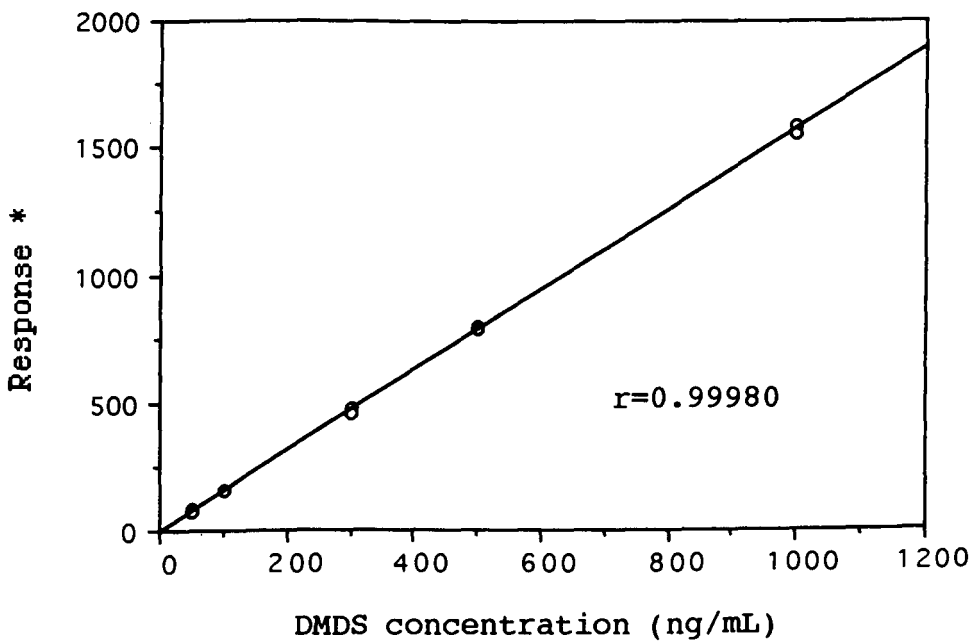

Figure 3.12 Calibration of DMDS

* Response = (peak area/internal standard area) X (internal standard height/peak height)$^{1/2}$

3.1.2 Development of the Solvent Extraction Method for Determination of Sulphur Compound Concentration

3.1.2.1 Solvent Extraction Method Using Extrelut®20

New whisky spirits normally contain 3-36 ng/mL of DMTS (Masuda and Nishimura, 1981; Leppänen *et al.*, 1983; Duncan, 1988) and less than 50 ng/mL of DMTS (Carter-Tijmstra, 1986). This range (3-36 ng/mL) of DMTS can not be detected by the direct injection method using a FPD. Therefore the solvent extraction and concentration method was required. To reduce sample preparation labour, Extrelut®20 was used for the extraction instead of the traditional solvent extraction method. Extrelut®20 has the following advantages:

a) Improvements on conventional liquid/liquid extraction

b) Lipophilic compounds are extracted by the organic solvent from the aqueous phase

c) The eluate does not contain emulsions

d) The eluate is ready for injection directly, or following concentration.

e) The pH of the aqueous solution to be extracted may be in the range from 1 to 13.

This extraction method is applied to lipophilic volatile compounds only. The some hydrophilic compounds may be removed from the eluate.

The range from 50 to 70% (v/v) ethanol concentration of samples was suitable for the recovery of the internal standard compound (di-n-decyl sulphide). However, the eluates from the samples of over 60% ethanol solution were sometimes difficult to concentrate to 0.1-0.5 mL, because the eluates contained a large portion of hydrophilic

compound (ethanol). Therefore, the ethanol concentration of the sample was adjusted to approximately 60% (v/v) for the recovery of the internal standard to improve the efficiency of the concentration of the eluate.

3.1.2.2 Calibration Graph by Solvent Extraction Method Using Extrelut®20

The calibration graph (Figure 3.13) was obtained with a 60% (v/v) alcoholic solution containing 0.1-50 ng/mL DMTS by the extraction method described in section 2.1.3. The same calculation method described in section 3.1.1.4 was used.

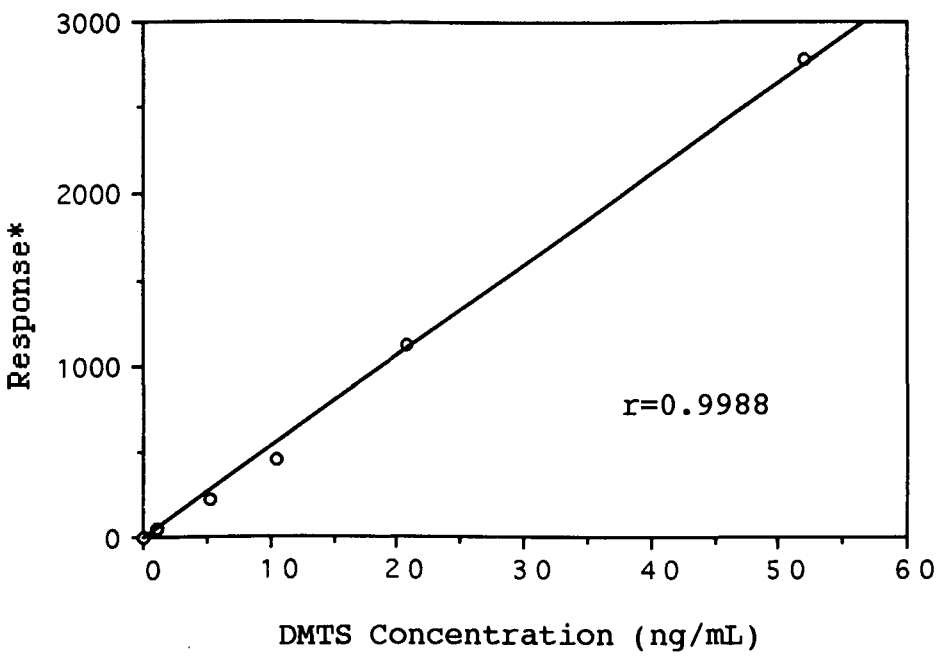

Figure 3.13 Calibration of DMTS

* Response = (peak area/internal standard area) X (internal standard height/peak height)$^{1/2}$

The correlation coefficient (0.9988) by this extraction method was slightly less than that of the direct injection method.

3.1.2.3 Reproducibility Solvent Extraction Method Using Extrelut®20

The reproducibility of this extraction method was evaluated using the standard solution containing 5.0 ng/mL of DMTS in 60% (v/v) ethanol solution. The coefficient of variation of the DMTS concentrations, using the extraction and concentration method, was 4.9% (Table 3.4). This result was satisfactory, as the coefficient of variation using capillary column gas chromatography is commonly less than 10% (Hoyt and Smith, 1991).

Table 3.4 Reproducibility of DMTS concentration analysed by Hewlett Packard gas chromatograph Model 5890 Series II equipped with FPD and using the extraction method

Run No.	DMTS (ng/mL)
1	5.26
2	4.63
3	5.09
4	4.81
5	5.35
Mean	5.03
SD	0.25
CV (%)	4.9

3.2 Thermal Reaction of Methional Without Buffer

After Ballance (1961) identified trace amount of DMDS after heating DL-methionine with ninhydrin, many reports of formation of DMDS from methionine have been published (Table 1.6). Yang et al. (1967) suggested that DMDS was formed from methionine via methional. Therefore, methional and methionine were investigated as the precursors of DMDS and DMTS under conditions simulating whisky distillation.

3.2.1 Effect of Duration of Distillation Experiment on the Formation of Sulphur Compounds from Methional

In general, the amount of volatile compounds formed increased both in number and quantity as either time or temperature of heating increased. Therefore, the effect of heating time on the formation of sulphur compounds from methional was investigated first.

As copper salt, 11.31 mg cupric acetate monohydrate (Aldrich), equivalent to 10 μg/mL Cu(II), was added to the reaction mixture (section 2.3.2). The reaction mixture was distilled as described in section 2.3.4. The reaction time, calculated from turning on the heat source to completion of distillation, varied between 26 and 55 min by adjusting the level of heating. The DMS, DMDS and DMTS concentrations in the distillates were measured (sections 2.1.2.1 and 2.1.3).

DMDS and DMTS but not DMS were detected. With an increase in reaction time, DMDS level in the distillate increased exponentially. The level of DMDS at 55 min was double at 49 min (Figure 3.14). However, there was no such rapid increase in DMTS formation (Figure 3.15).

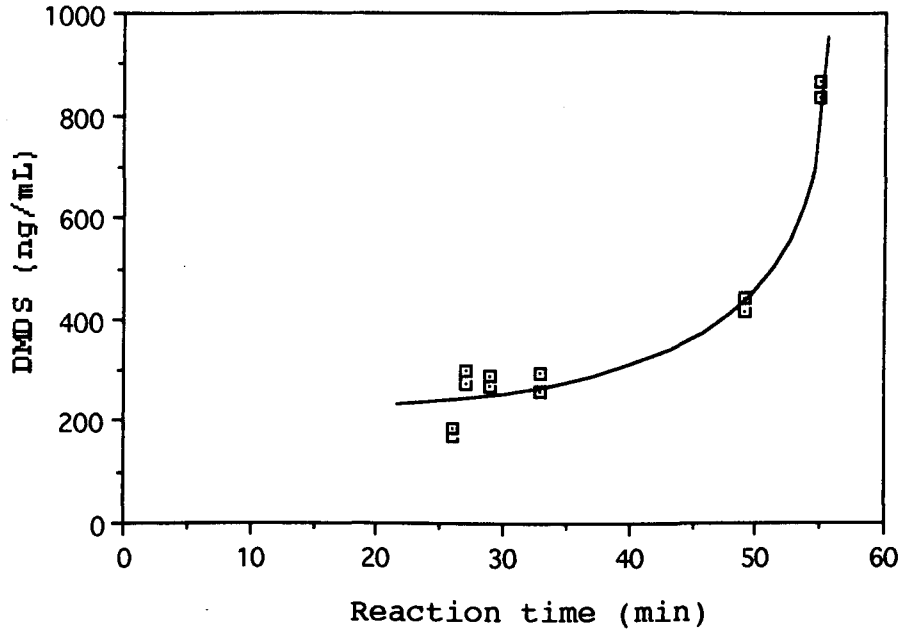

Figure 3.14 Effect of reaction time on DMDS formation from methional with cupric ion

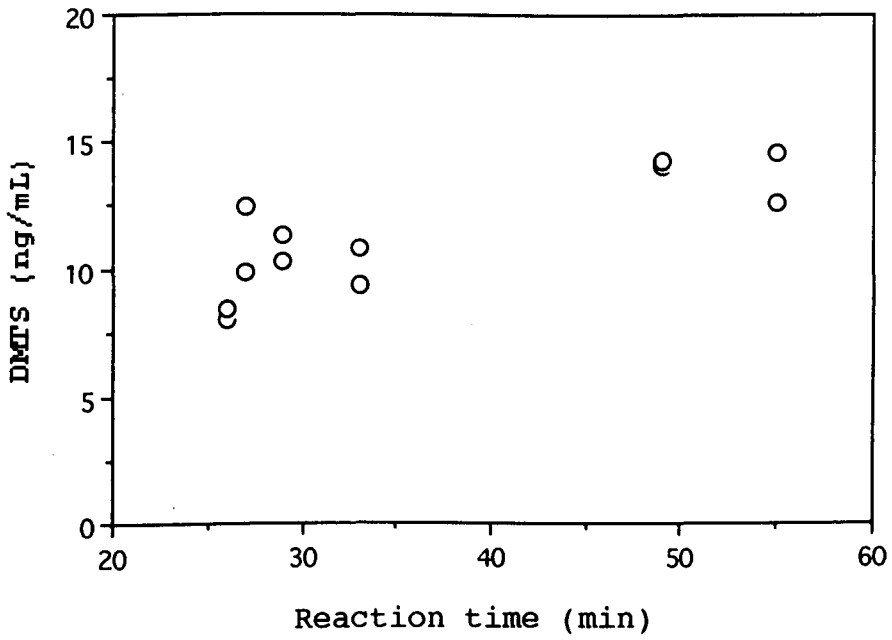

Figure 3.15 Effect of reaction time on DMTS formation from methional with cupric ion

3.2.2 Effect of Cupric ion Concentration on Formation of Sulphur Compounds from Methional

Copper is known to be involved in the formation of DMDS in wash distillation. Therefore the effect of quantity of cupric ion on the formation of sulphur compounds from methional was investigated.

Five different amounts (0, 15.7, 157, 785 and 1570 μM) of $CuAc_2$, equivalent to 0, 1.0, 10, 50, and 100 μg/mL Cu(II), were added to the reaction mixture (section 2.3.2). Each reaction mixture was distilled as described in section 2.3.4. DMS, DMDS and DMTS concentrations in the distillates were measured (sections 2.1.2.1 and 2.1.3).

DMS was not detected in any of the reaction mixtures. Formation of DMDS and DMTS increased with an increasing to addition of cupric ion up to 50 μg/mL. However, formation of DMDS and DMTS did not increase with concentrations over 50 μg/mL cupric ion (Figures 3.16 and 3.17).

After distillation there was a black precipitate on the boiling stone and inside the glassware. When this precipitate was dissolved in hydrochloric acid, the colour of the solution changed to yellow. The reactions of five known copper with HCl (Table 3.5) suggested that the black precipitate was CuS.

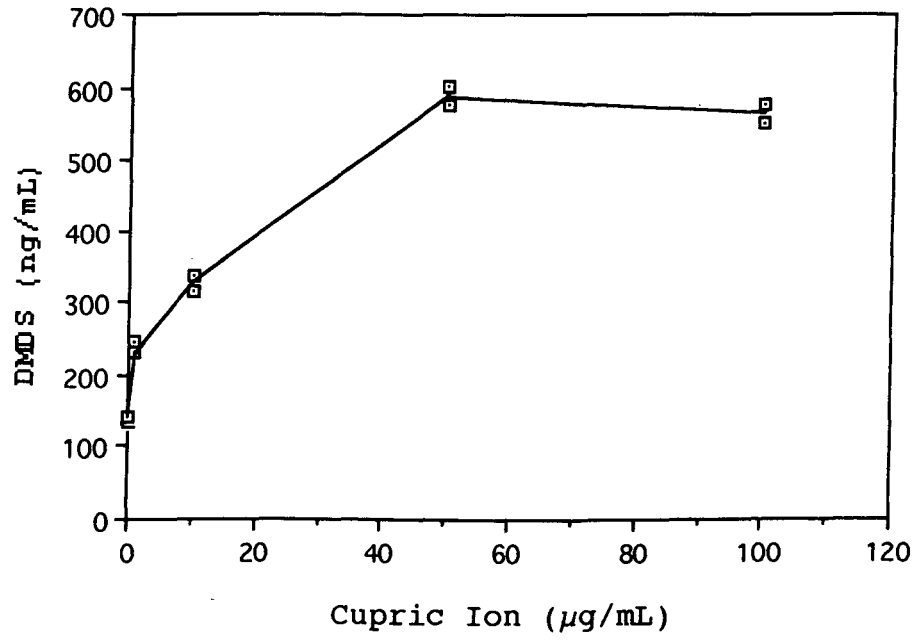

Figure 3.16 Effect of cupric ion concentration on DMDS formation from methional

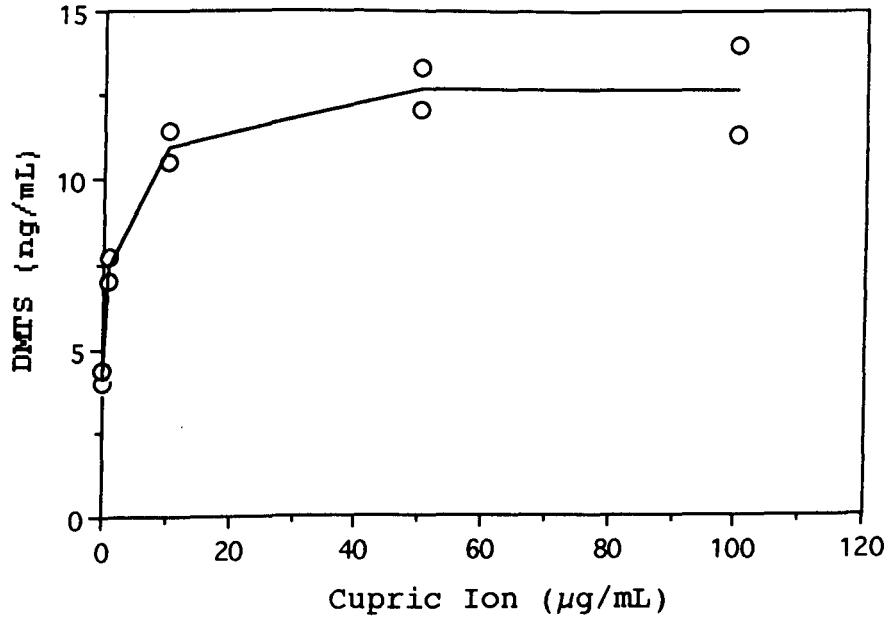

Figure 3.17 Effect of cupric ion concentration on DMTS formation from methional

Table 3.5 Solubility of copper salts into HCl and solution colour

Copper salt	Colour of compound	Colour of HCl sol.
Cu_2S	black	clear
CuS	black	yellow
CuCl	yellow-green	yellow
$CuCl_2$	ochre	yellow-blue
CuO	black	yellow

A black precipitate after distillation appears to be CuS as the results of solution colour of copper salts. Some of cupric ion bonded the sulphur atom decomposed from methional.

3.2.3 Effect of Copper Salts on the Formation of Sulphur Compounds from Methional

The effect of cupric salts on the formation of sulphur compounds from methional was investigated.

Seven copper salts, Cu_2S, CuS, CuCl, $CuCl_2$, CuO, $CuSO_4 \cdot 5H_2O$ (Aldrich) and $Cu(CH_3COO)_2 \cdot H_2O$, equivalent to 10 μg/mL (157 μM) Cu(I) or Cu(II), were added to the reaction mixture (section 2.3.2). Each reaction mixture was distilled (section 2.3.4). DMS, DMDS and DMTS concentrations in the distillates were measured (sections 2.1.2.1 and 2.1.3). The results are shown in Figures 3.18 and 3.19.

DMS was not detected in any of the reaction mixtures. CuCl, $CuCl_2$ and $CuSO_4$ did not affect DMDS formation. As with DMDS formation, $CuCl_2$ and $CuSO_4$ did not affect DMTS formation. CuCl affected DMTS formation as much as CuS.

Conversely, CuO did not affect DMTS formation in spite of a positive effect on DMDS formation. $CuAc_2$ most strongly affected both DMDS and DMTS formation from methional (Figures 3.18 and 3.19).

Cupric ion concentration of the reaction mixture before and after the distillation was measured (section 2.2). The results are shown in Figures 3.20 and 3.21. As $CuCl_2$, $CuSO_4$ and $CuAc_2$ have high solubilities in water, cupric ion levels of these reaction mixtures before distillation were almost equivalent to the added amounts. As CuCl has low solubility into water (solubility product constant = 1.9×10^{-7} at 25°C), cupric ion level was one third of added cuprous salt. Cuprous ion was oxidised to cupric ion in the solution, as cuprous ion is unstable. Conversely, CuS, Cu_2S and CuO have very low solubilities, cupric ion levels of these reaction mixture were nearly zero.

For $CuCl_2$ and $CuSO_4$, cupric ion levels after distillation were equivalent to added amounts in considering of liquid volume reduction by distillation. For $CuAc_2$, cupric ion level after distillation was slightly decreased. However, for CuCl, it was slightly increased. Before distillation, for CuS, Cu_2S and CuO, cupric ion levels were nearly zero.

The pH of the reaction mixtures before and after the distillation are shown in Figures 3.22 and 23.

In case of CuCl, $CuCl_2$ and $CuSO_4$, the pH of reaction mixtures before distillation were equivalent to no addition of copper salt. In the other case, pH levels of solution were a little higher. pH of the reaction mixtures after distillation were increased except $CuAc_2$.

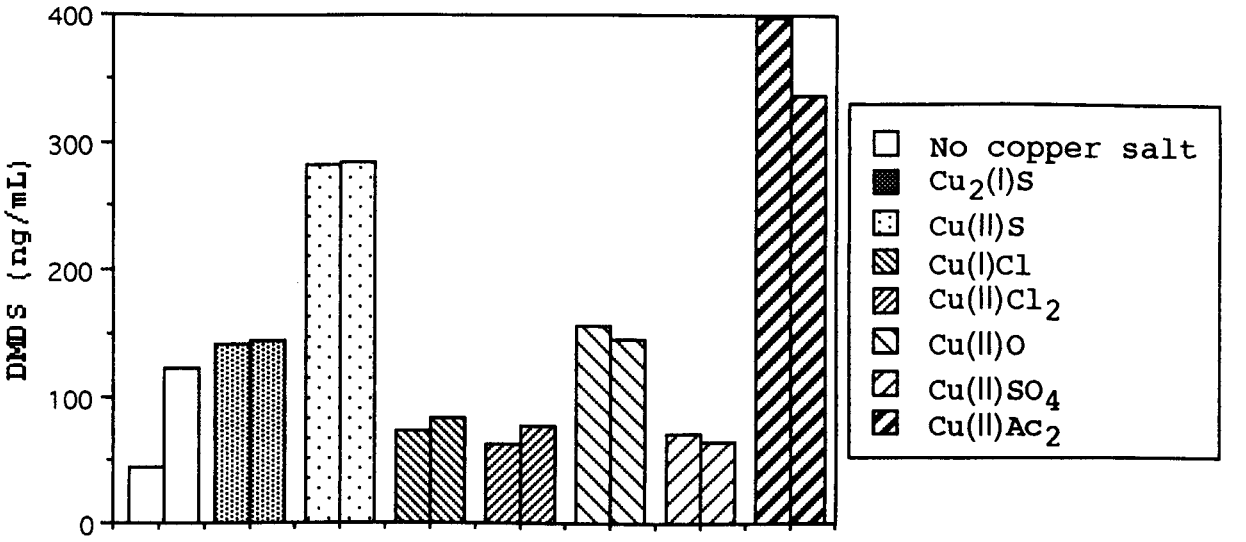

Figure 3.18 Effect of copper salts on DMDS formation from methional

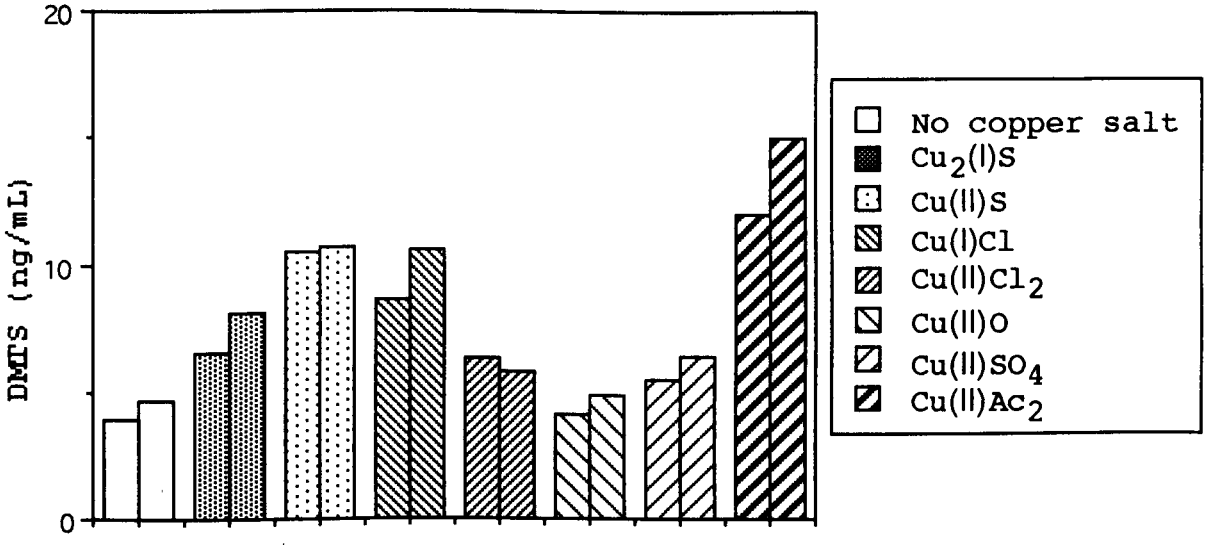

Figure 3.19 Effect of copper salts on DMTS formation from methional

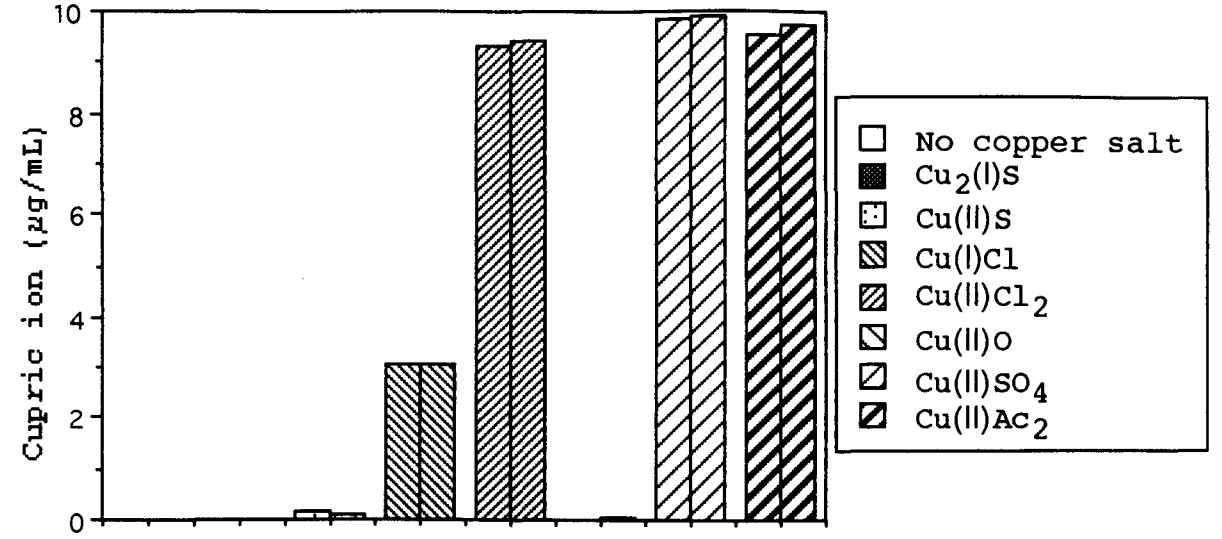

Figure 3.20 Cupric ion concentration of reaction mixture before distillation

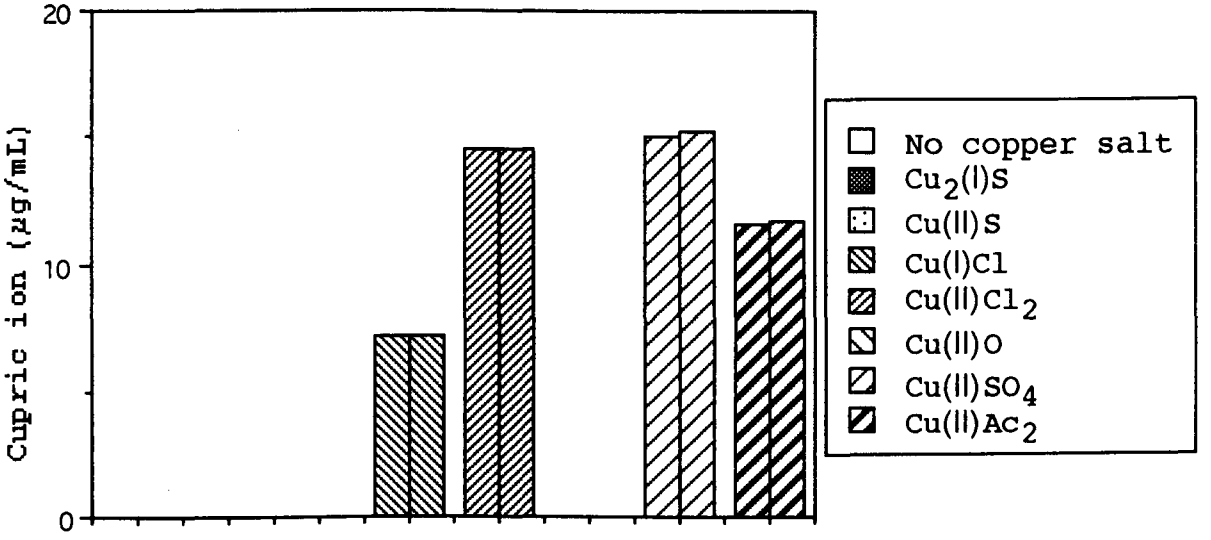

Figure 3.21 Cupric ion concentration of reaction mixture after distillation

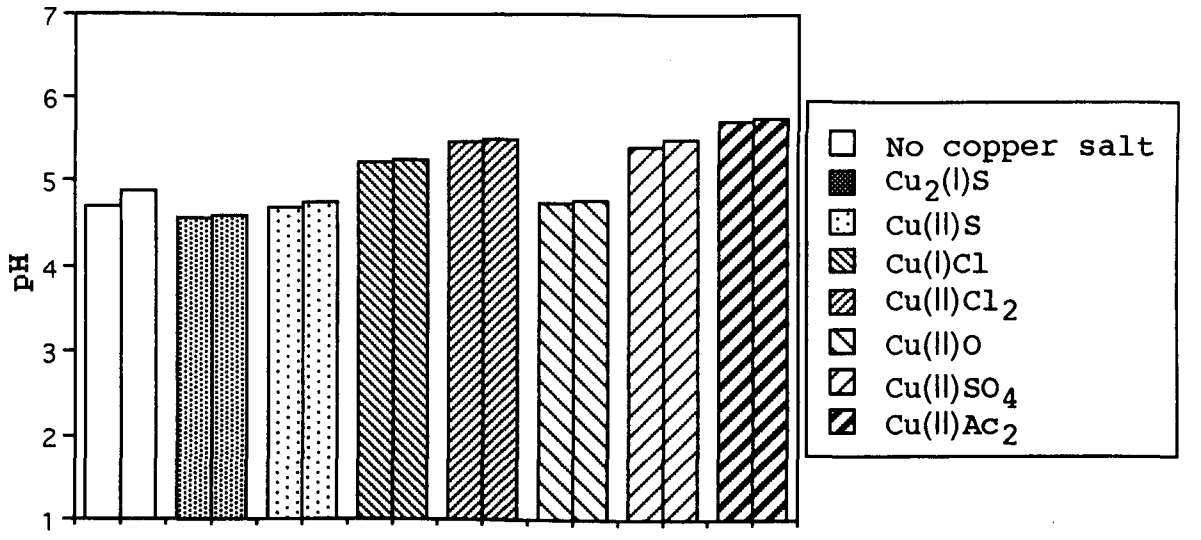

Figure 3.22 pH of reaction mixture before distillation

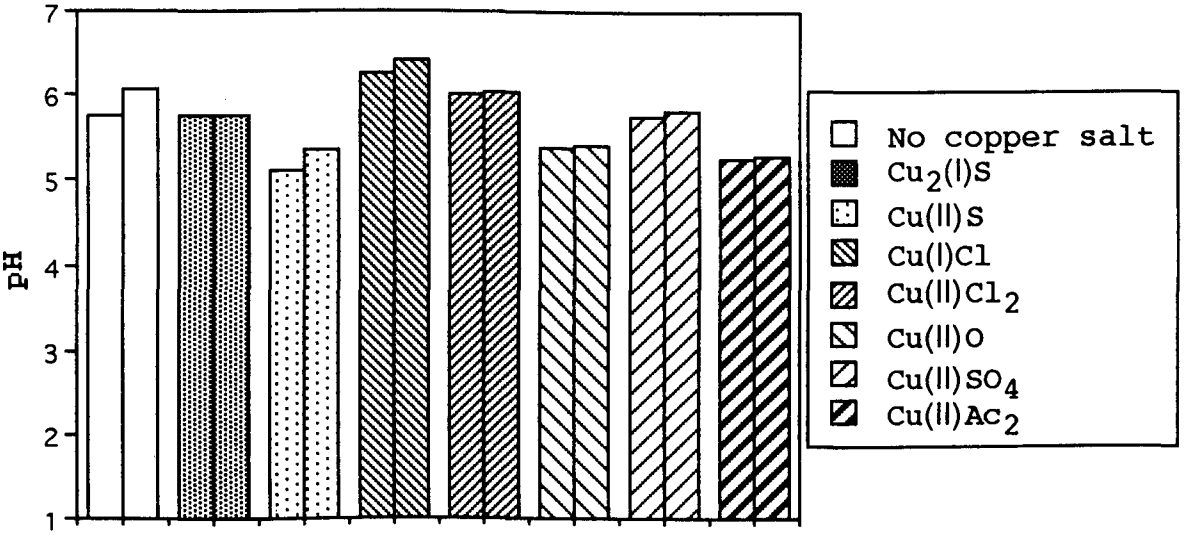

Figure 3.23 pH of reaction mixture after distillation

3.2.4 Effect of EDTA on the Formation of Sulphur Compounds from Methional with Cupric Ion

It was hypothesised that cupric ion which is surrounded by anions cannot increase the formation of sulphur compounds (Section 4.1.3). EDTA is a well-known chelating agent and can easily complex with cupric ion. Therefore, inhibitor effect of EDTA was investigated.

Three distillations were carried out each in duplicate to evaluate the effect of EDTA on the formation of sulphur compounds from methional. During one experiment copper ion was not added to the reaction mixture (section 2.3.2). To the others, 11.31 mg CuAc2, equivalent to 10 μg/mL Cu(II), with and without an equimolar concentration of EDTA was added. Each reaction mixture was distilled (section 2.3.4). DMS, DMDS and DMTS concentrations in the distillates were measured (sections 2.1.2.1 and 2.1.3).

DMS was not detected in any of the reaction mixtures. Although addition of cupric ion to the reaction mixture increased the formations of both DMDS and DMTS from methional, EDTA inhibited this cupric ion effect (Figures 3.24 and 3.25).

The cupric ion concentration of the reaction mixture before and after distillation was measured (section 2.2). The results are shown in Figures 3.26 and 3.27.

When cupric acetate alone was added, the cupric ion level before distillation was almost 10 μg/mL. When cupric acetate plus EDTA were added, the cupric ion level before distillation was only 10% of the added copper salt. As equimolar amounts copper salt and EDTA were added, 10% cupric ion was not chelated with EDTA.

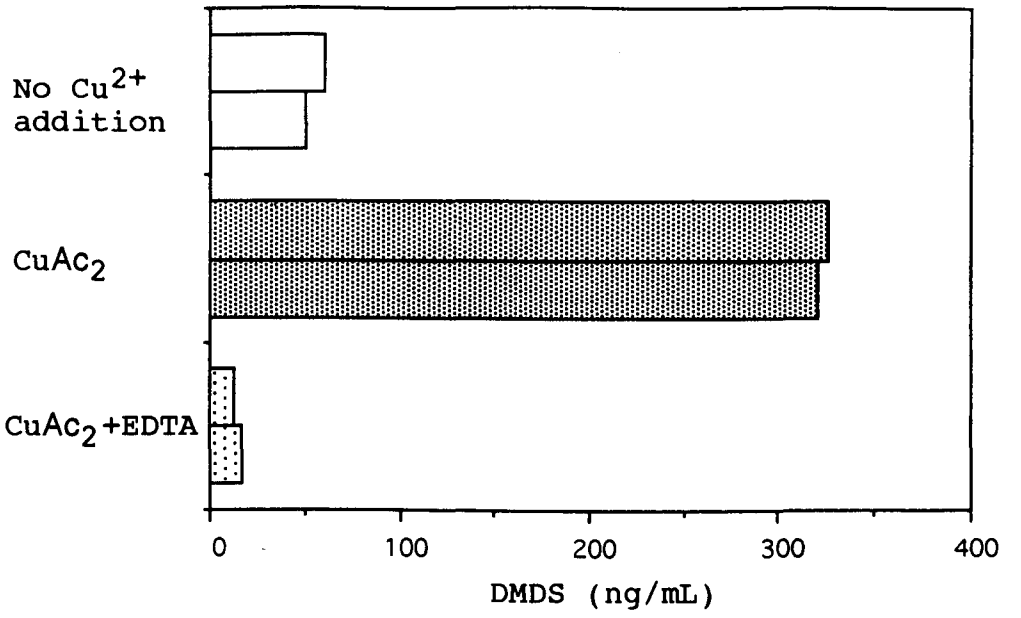

Figure 3.24 Effect of EDTA on DMDS formation from methional

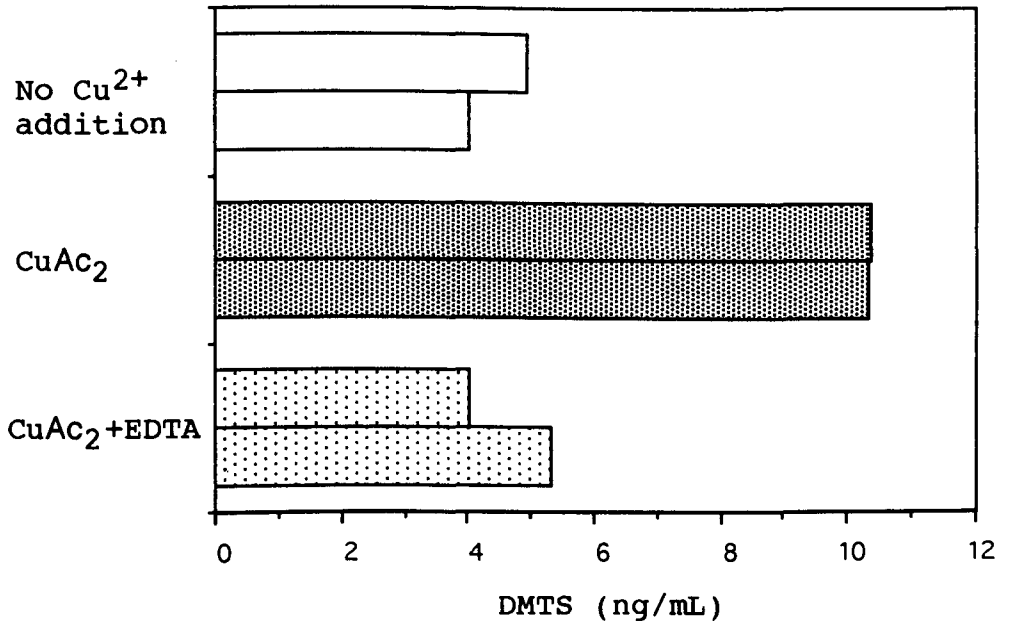

Figure 3.25 Effect of EDTA on DMTS formation from methional

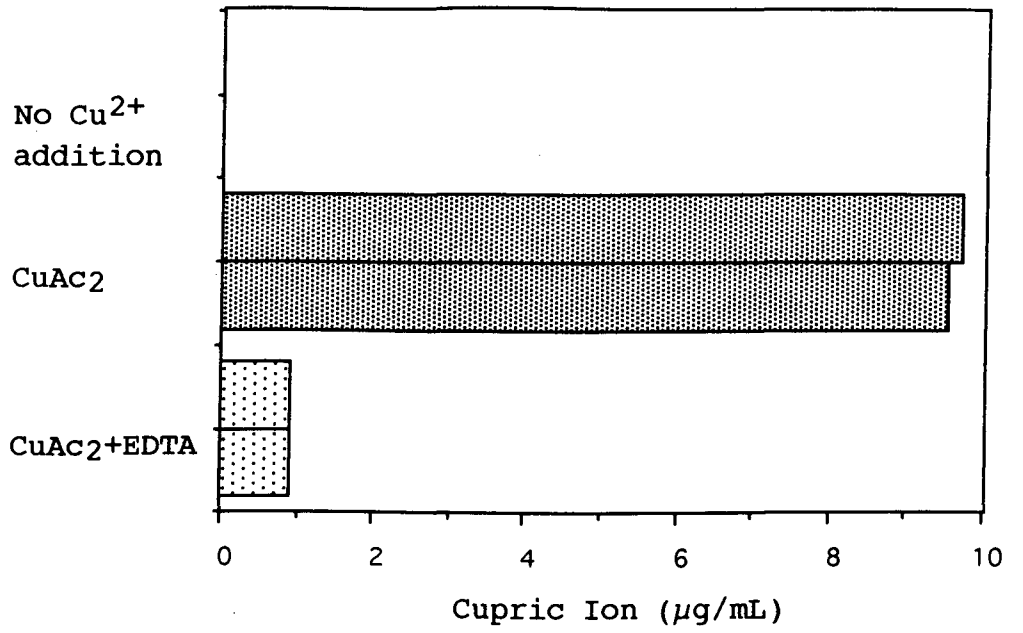

Figure 3.26 Cupric ion concentration of reaction mixture before distillation

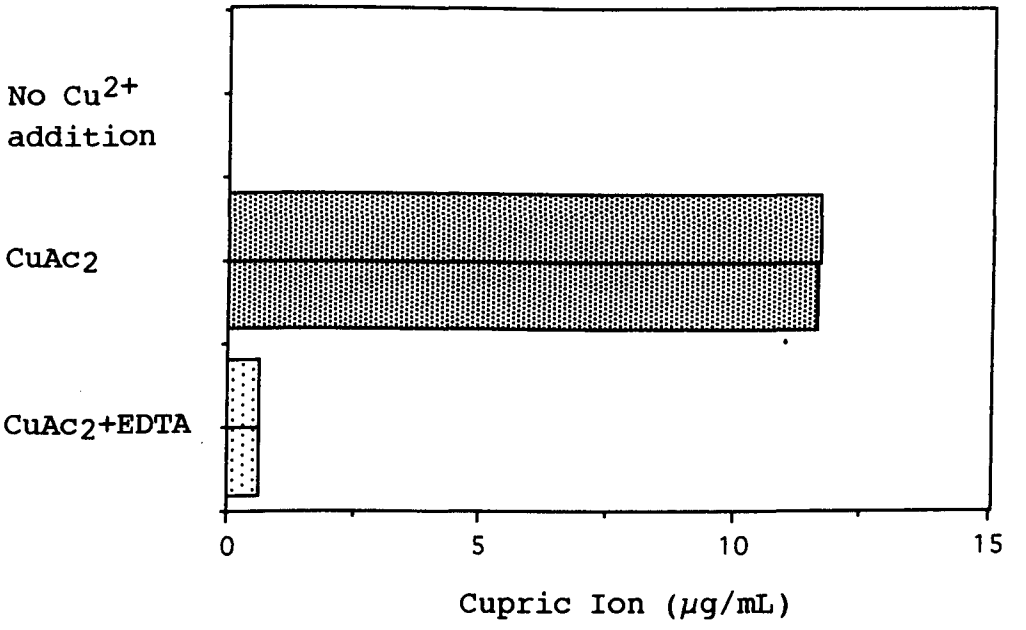

Figure 3.27 Cupric ion concentration of reaction mixture after distillation

When cupric acetate alone was added, cupric ion level after distillation was slightly decreased in considering of liquid volume reduction by distillation. When cupric acetate plus EDTA were added, the cupric ion level after distillation was lower than before distillation. During distillation, free cupric ion was chelated with EDTA.

The changes of pH by distillation are shown in Table 3.6. When either cupric salt was not added or cupric acetate only was added, the changes of pH agreed with the previous results described in section 3.2.3. When cupric acetate plusE DTA were added, the pH of the reaction mixtures were lower than the other examples because EDTA is a HCl salt. The pH of the reaction mixtures were slightly increased during distillation.

Table 3.6 pH of reaction mixture before and after distillation

	Before distillation		After distillation	
Run No.	1	2	1	2
No Cu^{2+} addition	4.90	4.70	6.07	5.75
$Cu(CH_3COO)_2$	5.73	5.75	5.26	5.28
$Cu(CH_3COO)_2$+EDTA	3.96	4.00	4.07	4.08

3.2.5 Effect of Aerobic and Anaerobic Conditions on the Formation of Sulphur Compounds from Methional

Oxygen has been suggested to participate in the formation of DMDS from methanethiol which is formed from the methionine decomposition. Therefore, the effect of aerobic

and anaerobic conditions on the formation of sulphur compounds from methional was investigated.

As copper salt, 11.31 mg $CuAc_2$, equivalent to 10 μg/mL Cu(II), was added to the reaction mixture (section 2.3.2). Three distillations were carried out. Two distillation was carried out with either O_2 or N_2 gas bubbling into the reaction mixture (section 2.3.4). Another distillation was carried out without any gas bubbling as a control to check the effect of gas bubbling on the concentration of sulphur compounds in the disttilate. Each reaction mixture was distilled (section 2.3.4). DMS, DMDS and DMTS concentrations in the distillates were measured (sections 2.1.2.1 and 2.1.3).

DMS was not detected in any of the reaction mixtures. Aerobic and anaerobic conditions did not affect the DMDS and DMTS formation from methional by using model distillation system (Figures 3.28 and 3.29).

Cupric ion concentration of the reaction mixture before and after distillation was measured (section 2.2). The results are shown in Figure 3.30. These results were the same as when using $CuAc_2$ alone as described in sections 3.2.3 and 3.2.4.

The pH of the reaction mixtures before and after distillation are shown in Figure 3.31. Under either aerobic, anaerobic or no control conditions the results were similar. After distillation, the pH of the reaction mixtures increased slightly by distillation. These results agreed with previous results for cupric acetate described in sections 3.2.3 and 3.2.4.

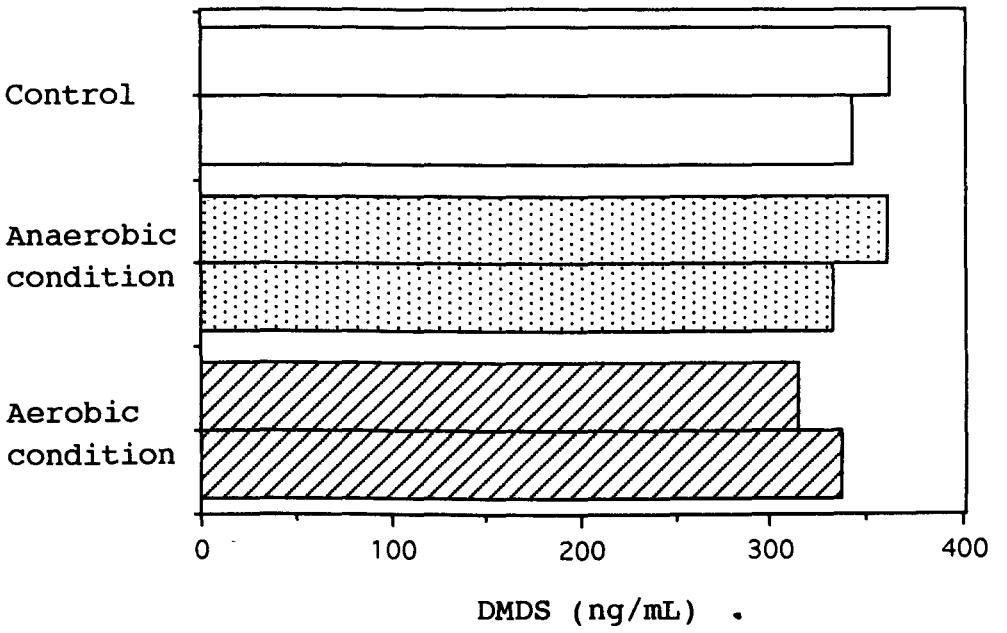

Figure 3.28 Effect of aerobic and anaerobic conditions on DMDS formation from methional

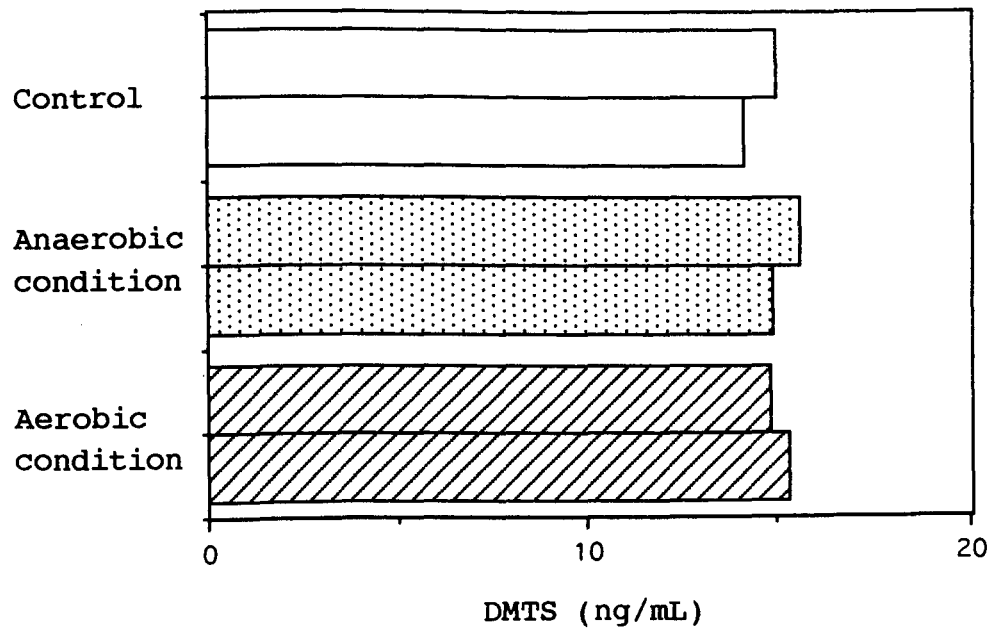

Figure 3.29 Effect of aerobic and anaerobic conditions on DMTS formation from methional

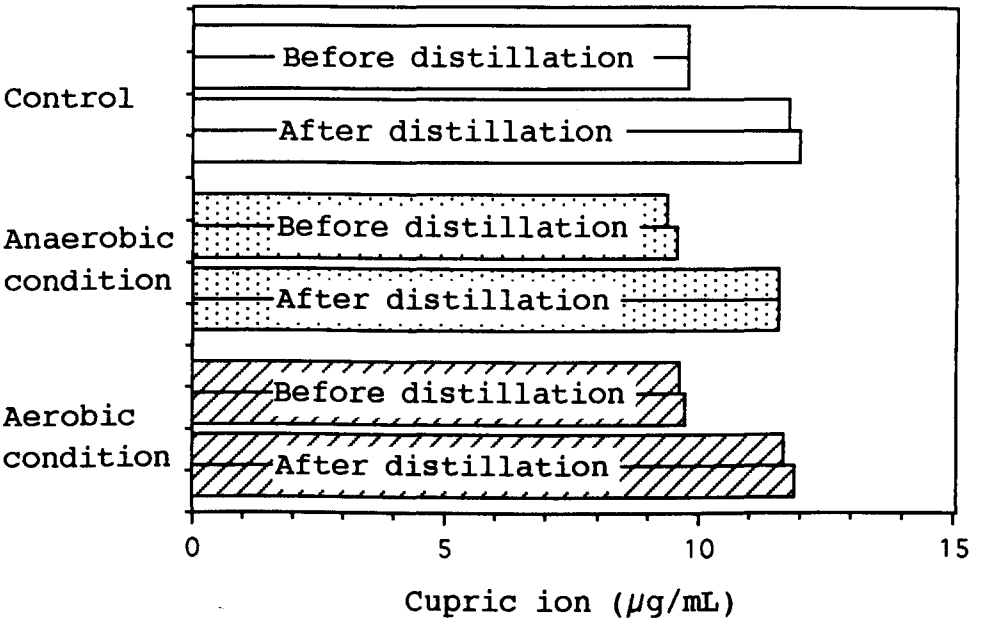

Figure 3.30 Cupric ion concentration of reaction mixture before and after distillation

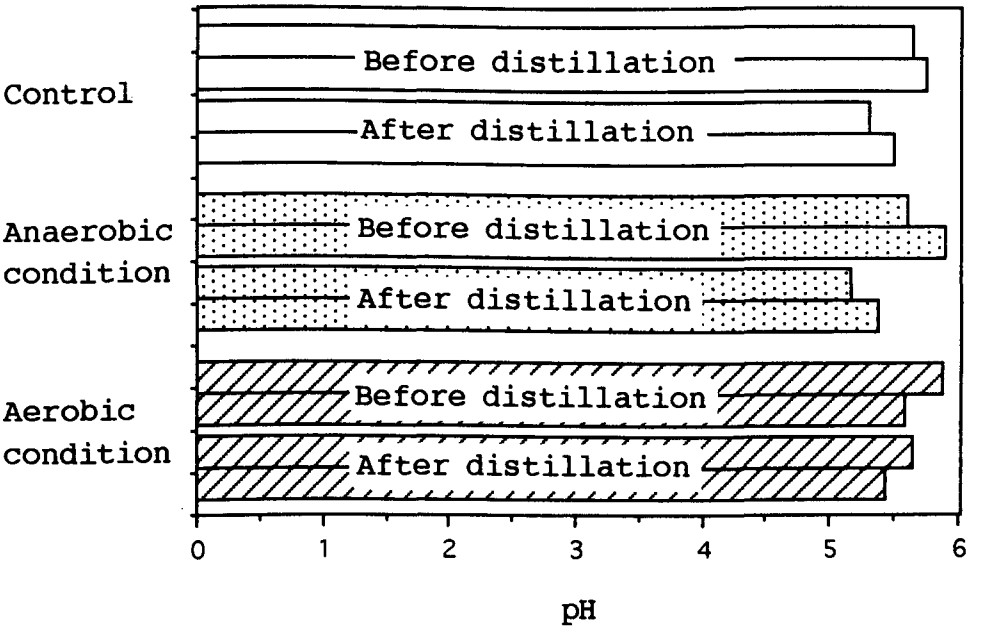

Figure 3.31 pH of reaction mixture before and after distillation

3.2.6 Decomposition of Methional by Reflux

The distillation time of wash still is normally 4-6 h in Scotch malt whisky distilleries. It was impossible to extend the reaction time more than 1 h using model distillation. Therefore, the trial of the decomposition of methional by the combination of model distillation and reflux, 6 h, was carried out

As copper salt, 11.31 mg $CuAc_2$, equivalent to 10 μg/mL Cu(II), was added to one reaction mixture (section 2.3.2). Another reaction mixture did not contain $CuAc_2$. These two reaction mixtures were refluxed in duplicate for 6 h to evaluate the effect of reflux condition on the formation of sulphur compound from methional using the model distillation equipment on which a reflux condenser was fitted. After reflux, the reaction mixture was cooled rapidly by ice. Then, each reaction mixture was distilled (section 2.3.4). DMS, DMDS and DMTS concentrations in the distillates were measured (sections 2.1.2.1 and 2.1.3).

DMS was not detected in any of the reaction mixtures. Addition of cupric ion to the reaction mixture did affect DMDS formation much more than no addition. Also, the DMDS concentration in the addition of cupric ion plus reflux was over 6 times than addition of cupric ion plus normal distillation only. However, DMDS concentration in the distillate without cupric ion was less than normal distillation (Figure 3.32). Conversely, cupric ion did not affect the DMTS formation under reflux in spite of a positive effect on DMTS formation (Figure 3.33).

Cupric ion concentration of the reaction mixture before and after reflux was measured as described in section 2.2. The results are shown in Table 3.7.

The cupric ion levels of reaction mixture after reflux were lower than before reflux. This result was different from that of cupric acetate only (sections 3.2.3, 3.2.4 and 3.2.5).

The pH of reaction mixtures before and after reflux are shown in Table 3.8. After reflux, the pH of reaction mixtures without cupric acetate slightly increased. Conversely, the pH of solutions with cupric ion decreased. These results were similar to previous results of cupric acetate described in sections 3.2.3, 3.2.4 and 3.2.5.

Table 3.7 Cupric ion of reaction mixture before and after reflux

(μg/mL)

	Before reflux		After distillation	
Run No.	1	2	1	2
No Cu^{2+} addition	N.D.	N.D.	N.D.	N.D.
$Cu(CH_3COO)_2$	9.6	0.8	8.3	8.7

Table 3.8 pH of reaction mixture before and after reflux

	Before reflux		After distillation	
Run No.	1	2	1	2
No Cu^{2+} addition	4.60	4.80	5.50	5.90
$Cu(CH_3COO)_2$	5.74	5.76	5.00	5.10

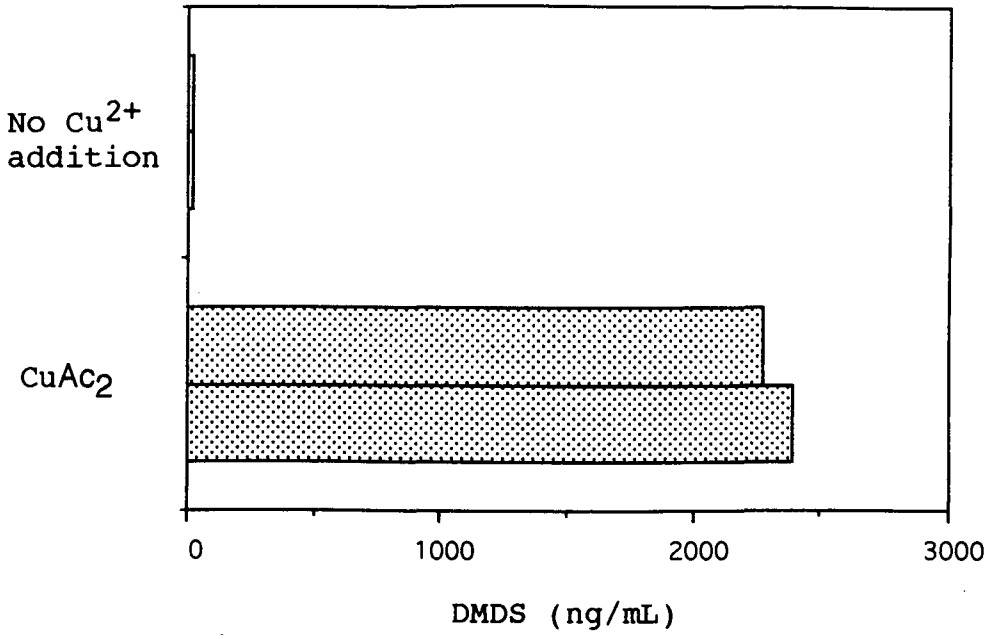

Figure 3.32 DMDS formation from methional by reflux with or without cupric acetate

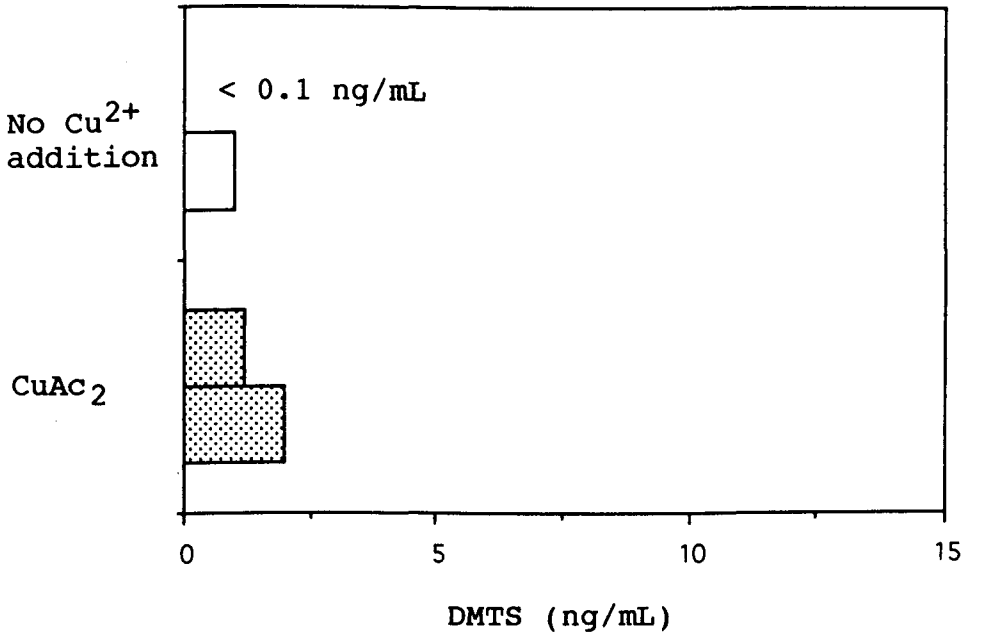

Figure 3.33 DMTS formation from methional by reflux with or without cupric acetate

3.3 Thermal Reaction of Methional with Buffer

The investigations were repeated in a buffered system, providing phosphate or sulphate ions as well as maximum stable pH.

3.3.1 Effect of pH on the Formation of Sulphur Compounds from Methional in Phosphate Buffer

Generally, the quantity of sulphur containing compounds increased with an increase of pH. Therefore, the effect of pH on the formation of sulphur compounds from methional was investigated with phosphate buffer.

3.3.1.1 Effect of pH on the Formation of Sulphur Compounds from Methional with and without Cupric Ion

Copper is known to be involved in the DMDS formation in wash distillation. The effect of pH on the formation of sulphur compounds from methional was evaluated in phosphate buffer with or without cupric ion.

The phosphate ion concentration in beer has been reported to vary over the range 260-995 μg/mL (Hough *et al.*, 1982). Therefore, an addition of 5 mM phosphate as the median value was used. With addition of copper salt, 11.31 mg $CuAc_2$, equivalent to 10 μg/mL Cu(II), was added to the reaction mixture (section 2.3.3). Each reaction mixture was distilled as described in section 2.3.4. DMS, DMDS and DMTS concentrations in the distillates were measured (sections 2.1.2.1 and 2.1.3). The results are shown in Figures 3.34, 3.35 and 3.36.

DMS was not detected in any of the reaction mixtures. Without cupric ion, DMDS formation from methional was minimal at lower pH (Figure 3.34). DMDS concentration at pH 8.0 was about 3 times as much as at pH 3.0. Cupric ion greatly accelerated DMDS formation at higher pH (Figure 3.35). DMDS concentration at pH 8.0 was about 63 times as much as at pH 3.0. On the other hand, cupric ion did not affect DMTS formation despite the effect on DMDS formation (Figure 3.36).

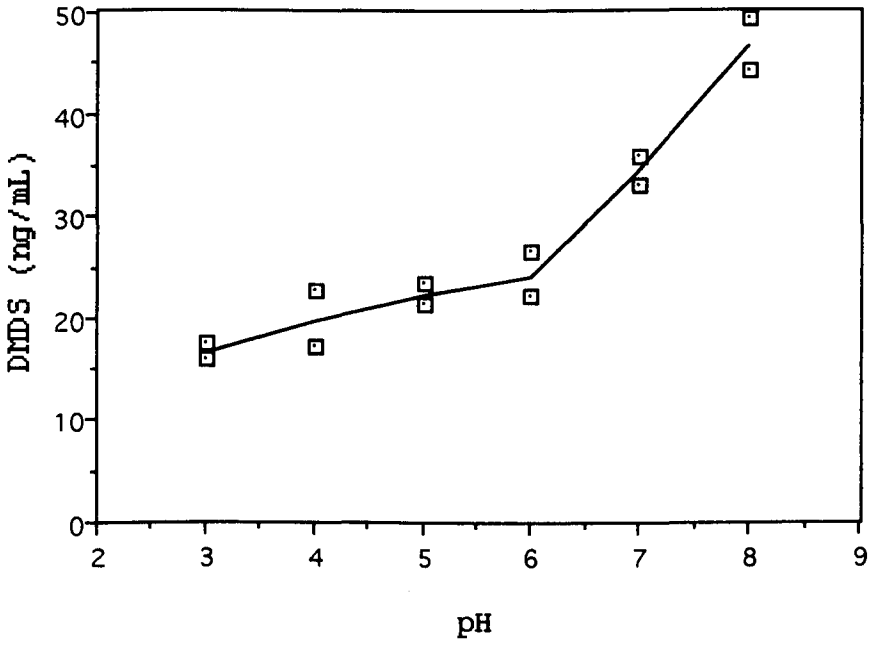

Figure 3.34 Effect of pH on DMDS formation from methional in phosphate buffer without cupric ion

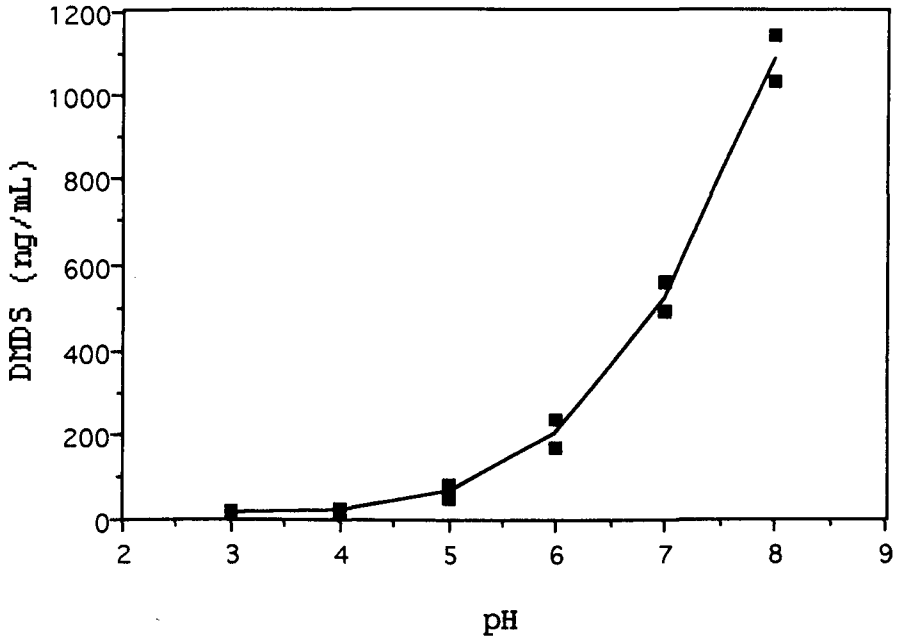

Figure 3.35 Effect of pH on DMDS formation from methional in phosphate buffer with cupric ion

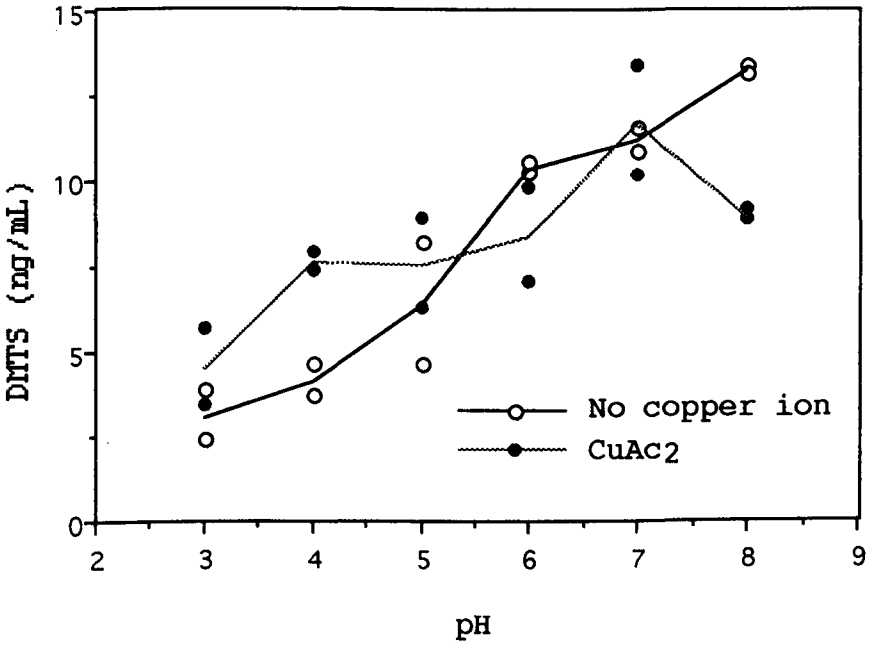

Figure 3.36 Effect of pH on DMTS formation from methional in phosphate buffer with or without cupric ion

3.3.1.2 Effect of pH on Cupric Ion Concentration in Reaction Mixtures before and after Distillation

Cupric ion concentration of the reaction mixture before and after distillation was measured as described in section 2.2. The results are shown in Figures 3.37 and 3.38. In Figure 3.38, the dotted line shows the concentration of cupric ion after distillation calculated from the concentration before distillation by considering the liquid volume reduction due to distillation.

In the presence of phosphate ion, the free cupric ion concentration in the reaction mixture before distillation was the same as with the added amounts between pH 3.0 and 4.0. With an increase in pH, the free cupric ion concentration in the reaction mixture decreased gradually from pH 4.0 to 6.0, and above pH 7.0 was below 3.0 μg/mL.

Between pH 3.0 and 8.0, the concentration of cupric ion did not change following distillation.

3.3.1.3 pH Changes of the Reaction Mixtures after Distillation with and without Cupric Ion

The pH changes of the reaction mixtures after distillation are shown in Figure 3.39. There was no pH change when the pH of the initial reaction mixture was between pH 3.0 and 4.0, although the pH of the mixture after distillation decreased slightly above pH 5.0. There was no major effect of cupric ion on the changes of pH.

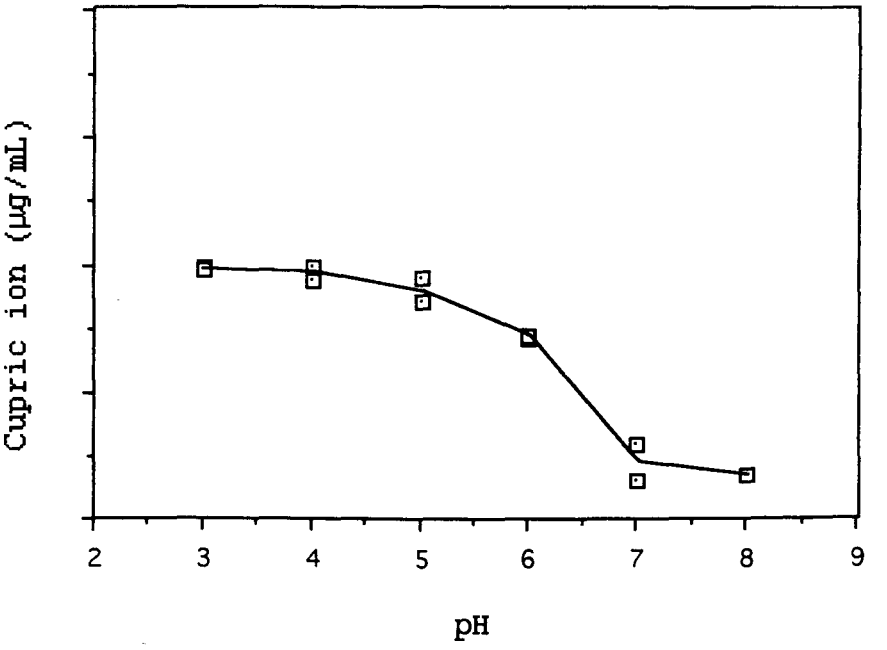

Figure 3.37 Cupric ion concentration of reaction mixture before distillation in phosphate buffer

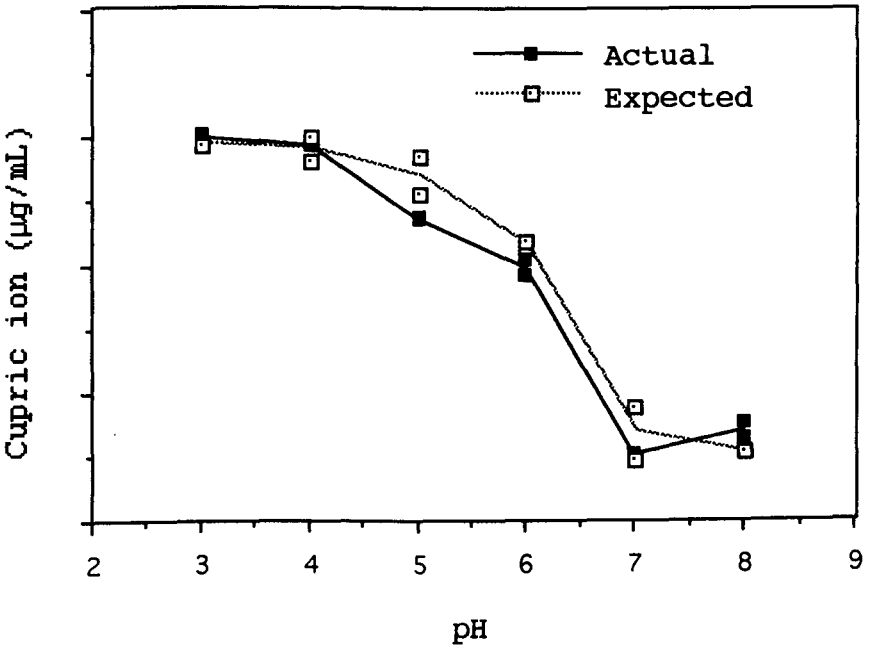

Figure 3.38 Cupric ion concentration of reaction mixture after distillation in phosphate buffer

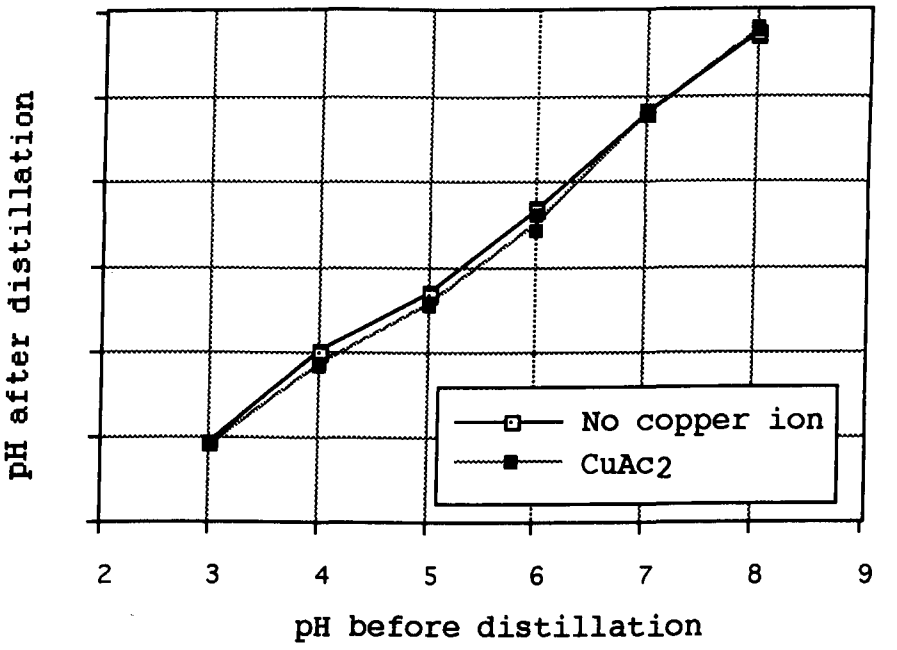

Figure 3.39 Change of pH following distillation in phosphate buffer

3.3.2 Effect of pH on the Formation of Sulphur Compounds from Methional in Sulphate Solution

In the same way as for phosphate buffer, the effect of pH on the formation of sulphur compounds from methional was investigated with sulphate.

3.3.2.1 Effect of pH on the Formation of sulphur compounds from Methional with and without Cupric Ion

Sulphate ion concentration in beer has been reported 150-400 μg/mL (Hough *et al.*, 1982). Therefore, an addition of 2mM sulphate as the median value was used. With addition of copper salt, 11.31 mg $CuAc_2$, equivalent to 10 μg/mL Cu(II), was added to the reaction mixture (section 2.3.3). Each reaction mixture was distilled as described in section 2.3.4. DMS, DMDS and DMTS concentrations in the distillates

were measured (sections 2.1.2.1 and 2.1.3). The results are shown in Figures 3.40, 3.41 and 3.42.

DMS was not detected in any of the reaction mixtures. Without cupric ion, DMDS formation from methional was maximal at pH 5.0 (Figure 3.40). DMDS concentration at pH 5.0 was about twice as much as at pH 3.0, 7.0 and 8.0. Cupric ion greatly accelerated DMDS formation at higher pH (Figure 3.41). DMDS concentration at pH 7.0 was about 100 times as much as at pH 3.0. On the other hand, cupric ion increased the formation of DMTS from methionine between pH 6.0 and 7.0 (Figure 3.42). Without cupric ion, DMTS formation from methional was maximal at pH 5.0. Whereas with cupric ion, DMTS formation was maximal at pH 7.0. The formation of DMDS and DMTS showed similar trends, although the absolute quantities are different.

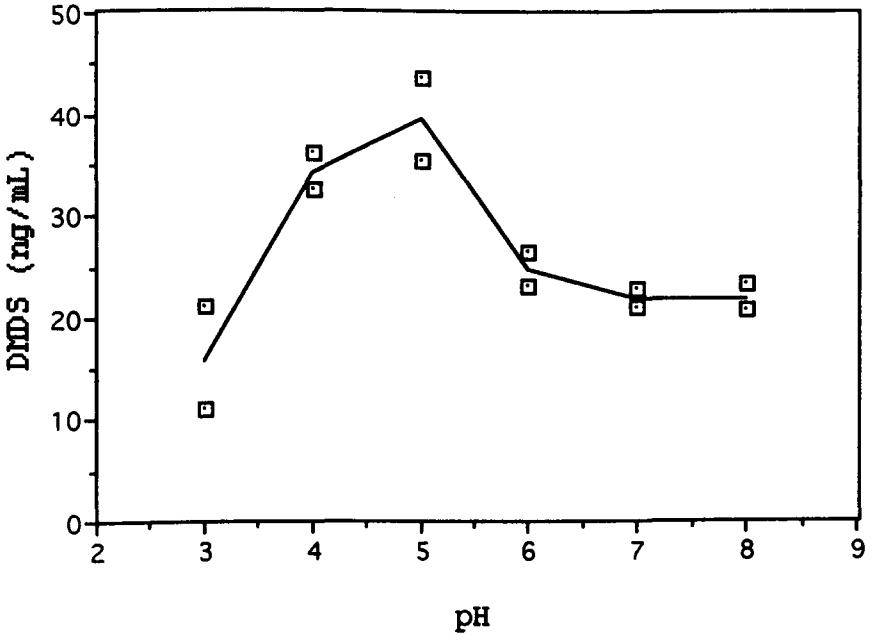

Figure 3.40 Effect of pH on DMDS formation from methional in sulphate solution without cupric ion

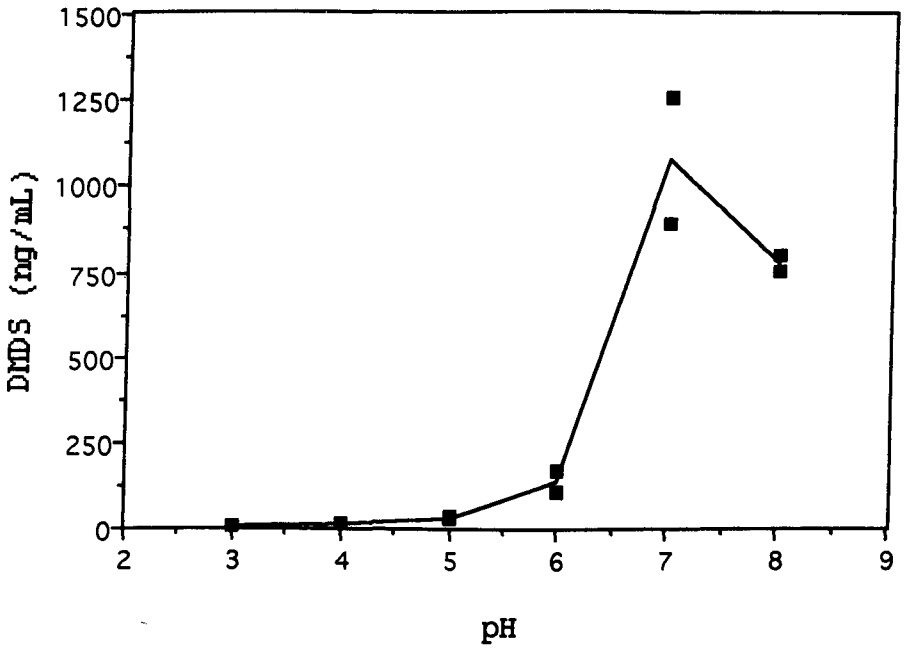

Figure 3.41 Effect of pH on DMDS formation from methional in sulphate solution with cupric ion

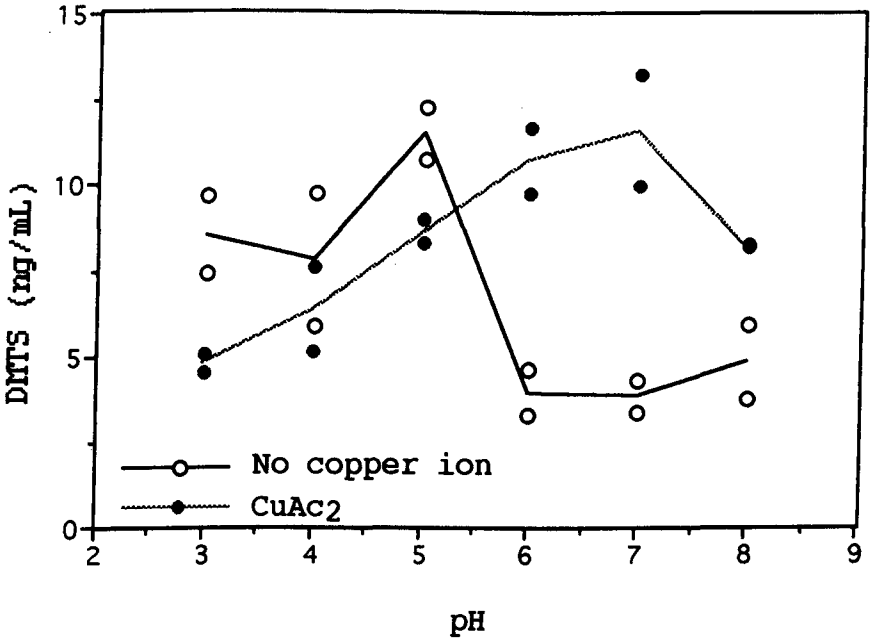

Figure 3.42 Effect of pH on DMTS formation from methional in sulphate solution with and without cupric ion

3.3.2.2 Effect of pH on Cupric Ion Concentration in Reaction Mixtures before and after Distillation

Cupric ion concentration of the reaction mixture before and after distillation was measured (section 2.2). The results are shown in Figures 3.43 and 3.44. In Figure 3.24, the dotted line shows the concentration of cupric ion after distillation calculated from the concentration before distillation by considering the liquid volume reduction due to distillation.

In presence of sulphate, the free cupric ion concentration in the reaction mixture before distillation was the same as with the added amounts between pH 3.0 and 6.0. The free cupric ion concentration in the reaction mixture decreased quickly from pH 7.0, and above pH 7.0, was below 2.0 μg/mL. Between pH 3.0 and 5.0, the concentration of cupric ion did not change following distillation . At pH 6.0, the detected cupric ion concentration was 70% of the theoretical value. The detected cupric ion was slightly lower than the theoretical value between 7.0 and 8.0.

3.3.2.3 pH Changing of Reaction Mixtures after Distillation with and without Cupric Ion

The pH change of the reaction mixtures after distillation are shown in Figure 3.45. There was no pH change when the pH of the initial reaction mixture was between pH 3.0 and 4.0 and at pH 7.0. Without cupric ion, pH of mixture after distillation increased slightly between pH 5.0 and 6.0, although it increased at pH 8.0. Conversely, with cupric ion, pH of mixture after distillation degreased between 5.0 and 7.0, although it increased at pH 8.0.

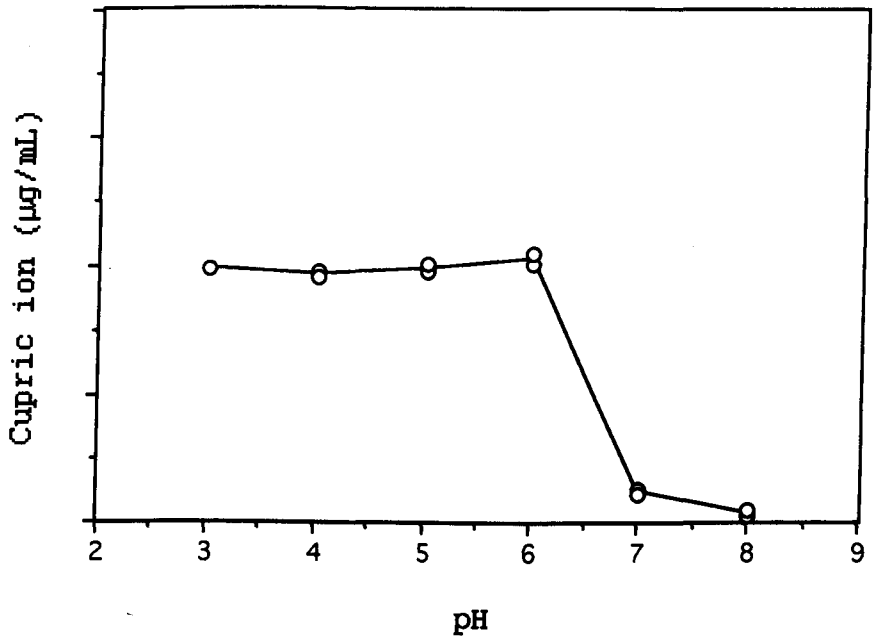

Figure 3.43 Cupric ion concentration of reaction mixture before distillation in sulphate solution

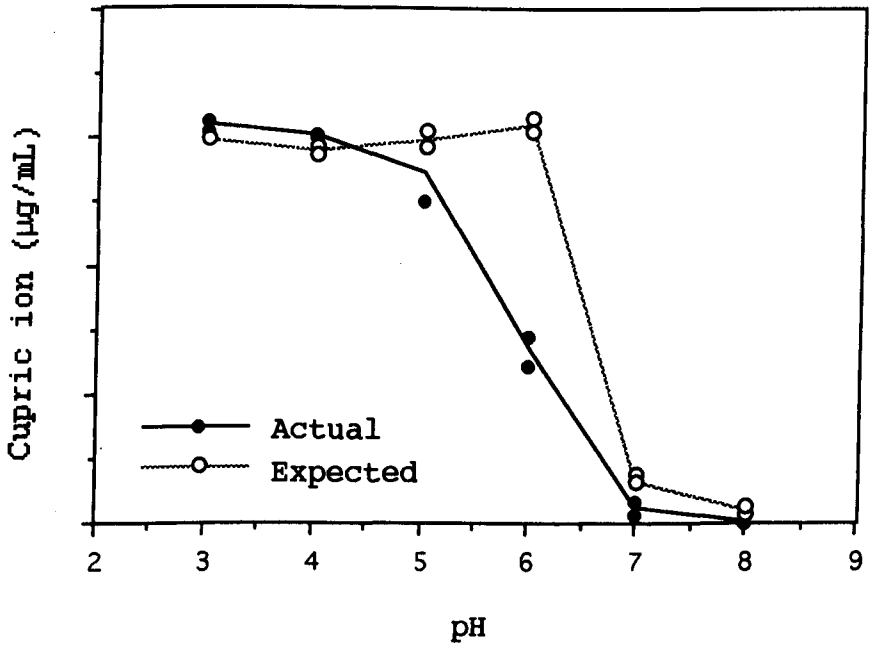

Figure 3.44 Cupric ion concentration of reaction mixture after distillation in sulphate solution

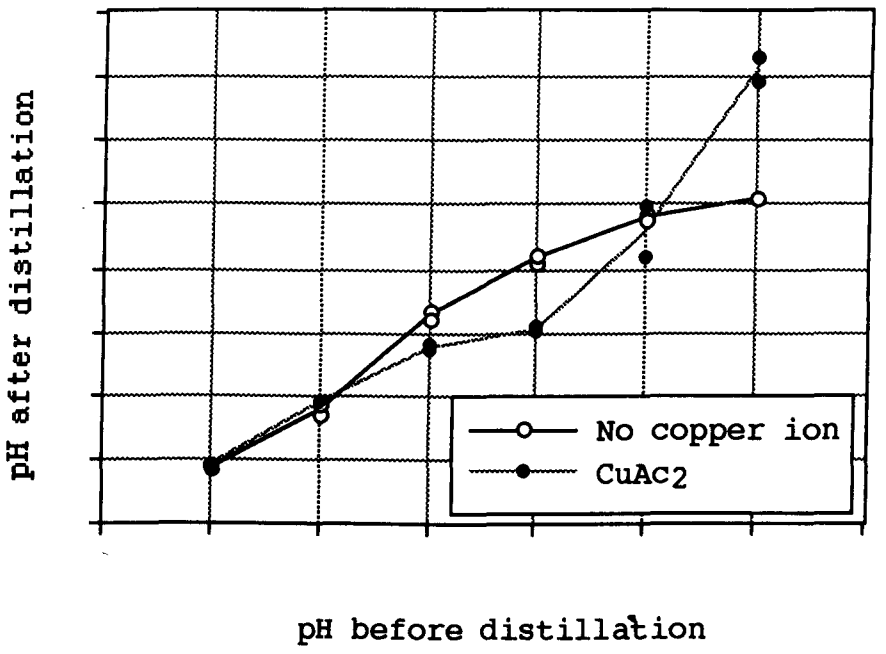

Figure 3.45 Change of pH following distillation in sulphate solution

3.4 Thermal Reaction of L-Methionine

Methionine was investigated as a possible precursor of DMDS and DMTS.

3.4.1 Using Model Distillation

3.4.1.1 Thermal Reaction of L-Methionine with H_2O_2 or L-Ascorbic Acid

H_2O_2 or L-ascorbic acid increased the ethylene formation from methionine using copper catalyst. Therefore, the effect of H_2O_2 or L-ascorbic acid on the formation of sulphur compounds from L-methionine was investigated.

Three distillations were carried out in duplicate to evaluate the effect of H_2O_2 and L-ascorbic acid on the formation of sulphur compounds from L-methionine. Methionine concentration in beer has been reported between 0 and 257μM (Hough *et al.*, 1982). Therefore an addition of 100μM methionine as the median value was used. As copper salt, 11.31 mg $CuAc_2$, equivalent to 10 μg/mL Cu(II), was added to the reaction mixture (section 2.3.2). Either 10 mM H_2O_2 (BDH) or 10 mM L-ascorbic acid (Sigma) was added to the reaction mixture. The other reaction mixture did not contain either H_2O_2 or L-ascorbic acid. Each reaction mixture was distilled as described in section 2.3.4. DMS, DMDS and DMTS concentrations in the distillates were measured (sections 2.1.2.1 and 2.1.3).

DMS was not detected in any of the distillate. Without H_2O_2 or L-ascorbic acid, neither DMDS nor DMTS was detected in the distillate. In present of H_2O_2, both DMDS and DMTS formation from L-methionine with cupric ion greatly

increased, although L-ascorbic acid has a slight effect (Figures 3.46 and 3.47).

Cupric ion concentration of the reaction mixture before and after reflux was measured as described in section 2.2. The results are shown in Table 3.9.

There was no difference between each samples. The levels of cupric ion before and after distillation were similar to that of cupric acetate only (sections 3.2.3, 3.2.4 and 3.2.5).

Table 3.9 Cupric ion concentration of reaction mixture before and after distillation

	Before distillation		After distillation	
Run No.	1	2	1	2
$Cu(CH_3COO)_2$	9.5	9.7	12.3	12.9
$Cu(CH_3COO)_2+H_2O_2$	9.6	10.0	11.8	12.6
$Cu(CH_3COO)_2$ +L-ascorbic acid	9.8	9.6	12.3	11.7

The pH of reaction mixtures before and after distillation are shown in Table 3.10. After distillation, the pH of each reaction mixture slightly decreased. This results nearly agreed with the previous results with cupric acetate described in sections 3.2.3, 3.2.4, 3.2.5 and 3.2.6. With L-ascorbic acid, the pH of the reaction mixtures before and after distillation are lower than the others.

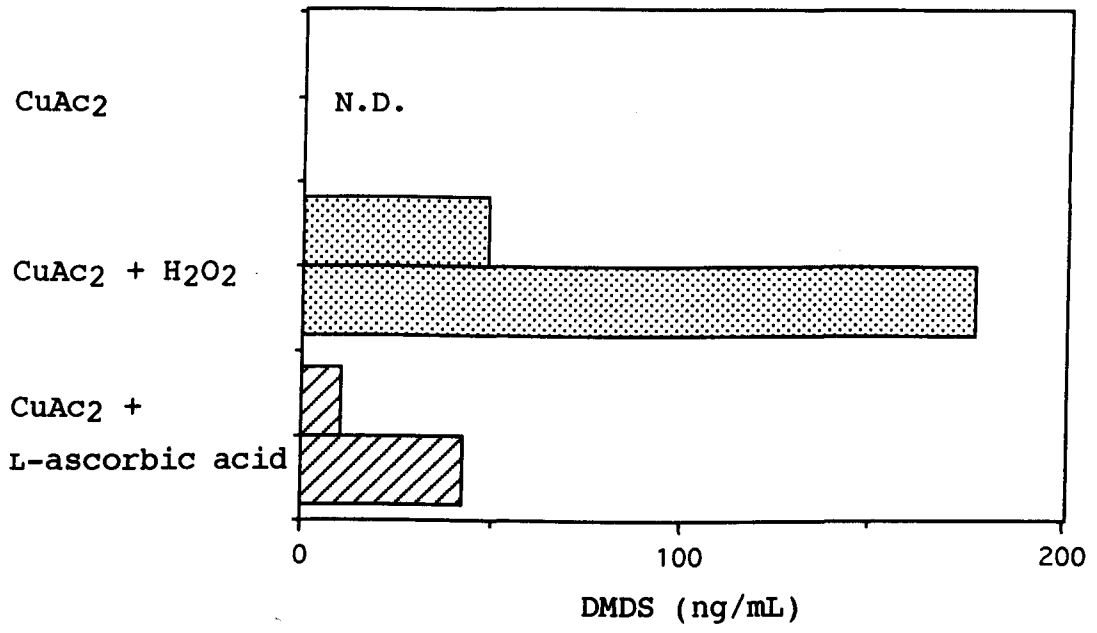

Figure 3.46 Effect of H_2O_2 and L-ascorbic acid on DMDS formation from L-methionine with cupric acid

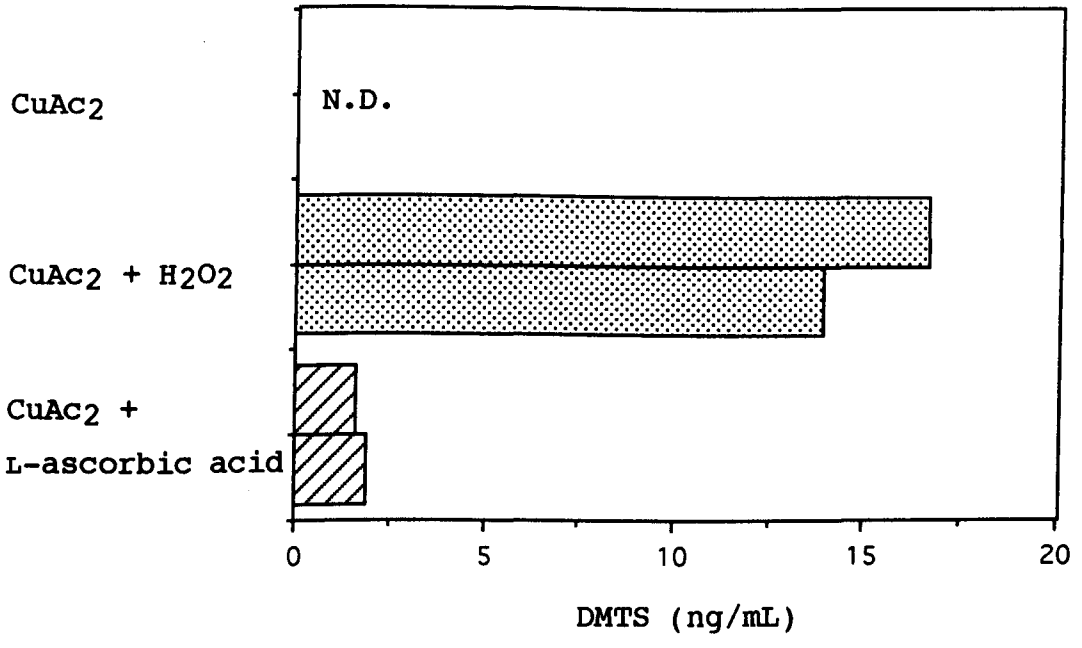

Figure 3.47 Effect of H_2O_2 and L-ascorbic acid on DMTS formation from L-methionine with cupric acid

Table 3.10 pH of reaction mixture before and after distillation

	Before distillation		After distillation	
Run No.	1	2	1	2
$Cu(CH_3COO)_2$	5.77	5.69	5.39	5.25
$Cu(CH_3COO)_2+H_2O_2$	5.55	5.57	5.19	5.20
$Cu(CH_3COO)_2$ +L-ascorbic acid	3.92	3.85	4.19	4.18

3.4.1.2 Effect of pH on the Formation of Sulphur Compounds from Methionine

Generally, the quantity of sulphur containing compounds increased with an increase of pH. Therefore, the effect of pH on the formation of sulphur compounds from L-methionine was investigated with phosphate buffer with or without cupric ion. Three reaction mixtures (Section 2.3.3) were distilled at each pH points (Section 2.3.4). A equimolar amount (100 μM) of D-glucose was added to two of them. $CuAc_2$ was added to one of the reaction mixtures in which D-glucose were added. DMS, DMDS and DMTS concentrations in the distillates were measured (sections 2.1.2.1 and 2.1.3).

DMS, DMDS and DMTS were not detected in any of the distillates.

Cupric ion concentration of the reaction mixture before and after distillation was measured as described in section 2.2. The results are shown in Figures 3.48 and 3.49. These results were similar to previous results with cupric acetate

in phosphate buffer described in section 3.3.1.2, although cupric ion concentration at pH 5.0 was higher.

The pH change of the reaction mixtures after distillation are shown in Figure 3.50. There were decreased slightly of pH at 4.0 and 7.0, although the pH of the mixture decreased about 0.5 between pH 5.0 and 6.0. There was no major effect of cupric ion and D-glucose on the changes of pH.

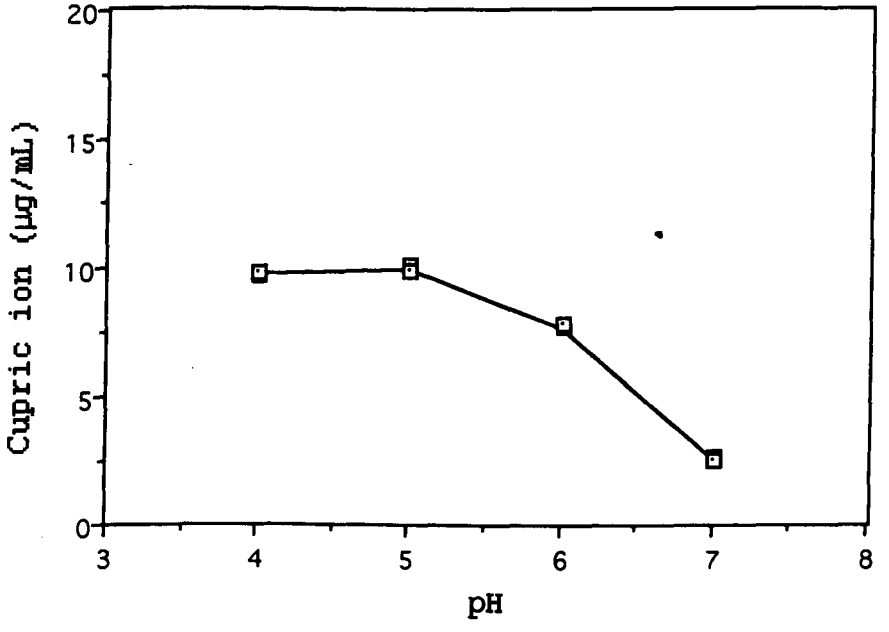

Figure 3.48 Cupric ion concentration of reaction mixture before distillation in phosphate buffer

3.4.2 Development of a Thermal Reaction System Using Sealed Vials

DMDS and DMTS were not detected from thermal reaction of L-methionine with or without cupric ion using model distillation system at any pH. Arroyo and Lillard did, however, detect DMS and DMDS on heating methionine in model system. Effect of heat on the amino acid was reconsidered.

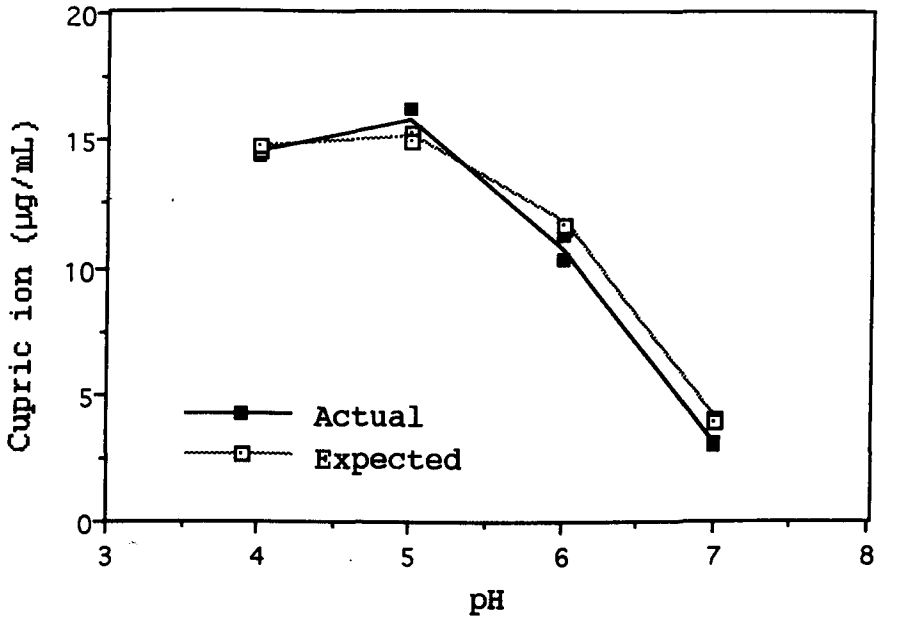

Figure 3.49 Cupric ion concentration of reaction mixture after distillation in phosphate buffer

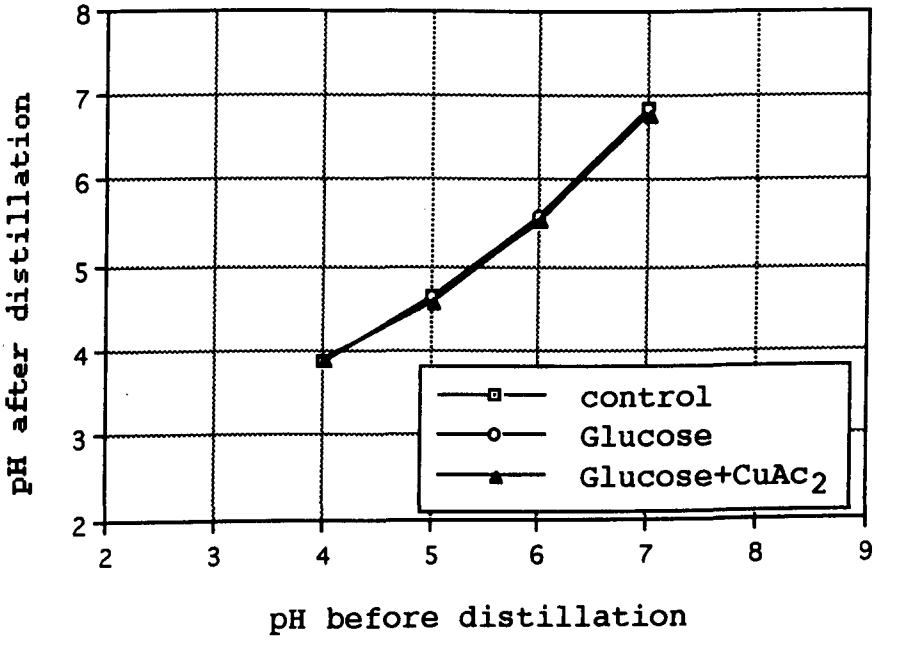

Figure 3.50 Change of pH follwoing distillation in phosphate buffer

3.4.2.1 Arroyo's Experiment

An experiment were conducted to evaluate the experimental conditions of Arroyo and Lilland. An equimolar amount (1.0 mM) of L-methionine or DL-methionine with and without 1.0 mM D-glucose was added to 11 mL distilled water in a 12 mL centrifuge tube which can be sealed. Reaction mixture was not buffer and the pH was not adjusted during reaction. The reaction mixture was heated for 2 h at 100 °C as described in section 2.3.3. DMS, DMDS, DMTS and methional concentrations in the distillates were measured (sections 2.1.2.1 and 2.1.3). The results are shown in Table 3.11.

DMTS was not detected in any of the reaction mixture. DMS was not confirmed due to the overlapping by another compound. DMDS and methional levels in both L-methionine and DL-methionine reaction mixtures with D-glucose were equivalent. DMDS was not detected in reaction mixture without D-glucose. Thermal reaction system using a sealed vial was used for the further decomposition of L-methionine instead of model distillation system to analyse methional concentration correctly in the reaction mixture because methional does not recover by distillation. In addition the reaction time can be changed easily using sealed vial system.

The pH of reaction mixtures in L-methionine and DL-methionine with D-glucose were 5.00 and 4.97 respectively. The pH without glucose was 4.95.

Table 3.11 DMDS and methional concentration and pH in the reaction mixture from L-methionine or DL-methionine with or without D-glucose

	DMDS (ng/mL)		Methional (μg/mL)		pH	
Run No.	1	2	1	2	1	2
L-Methionine + D-Glucose	129.5	114.1	64.5	80.9	5.00	5.01
DL-Methionine + D-Glucose	105.1	125.9	70.6	92.5	4.96	4.97
L-Methionine	N. D.	N.D.	3.0	3.6	4.95	4.94

N. D. : not detected

3.4.2.2 Effect of Aerobic and Anaerobic Conditions on the Formation of Sulphur Compounds from L-Methionine

Oxygen was suggested to participate the formation of DMDS from methanethiol which is formed from the methionine decomposition. Therefore, the effect of aerobic and anaerobic conditions on the formation of sulphur compounds from methional was investigated.

D-Glucose was added to the reaction mixtures as reducing sugar (section 2.3.5). The reaction mixture was heated as described in section 2.3.6. DMS, DMDS, DMTS and methional concentrations were measured (sections 2.1.2.1 and 2.1.3). The results are shown in Figures 3.51 and 3.52.

DMS was not detected due to the overlapping of peaks by another compound. DMTS was not detected in any of the reaction mixtures. Under aerobic conditions, the level of both DMDS and methional was higher than under the anaerobic

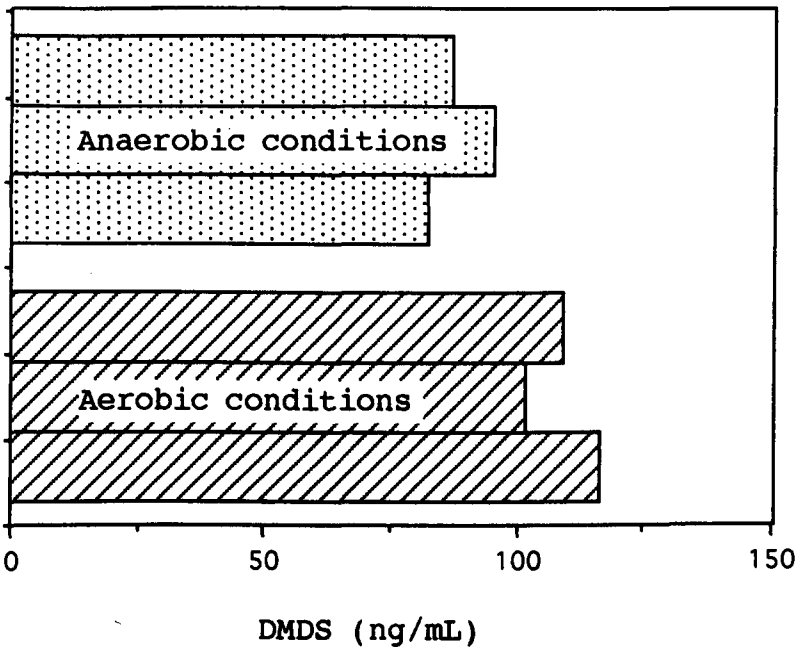

Figure 3.51 Effect of aerobic and anaerobic conditions on DMDS formation from L-methionine without cupric ion in phosphate buffer

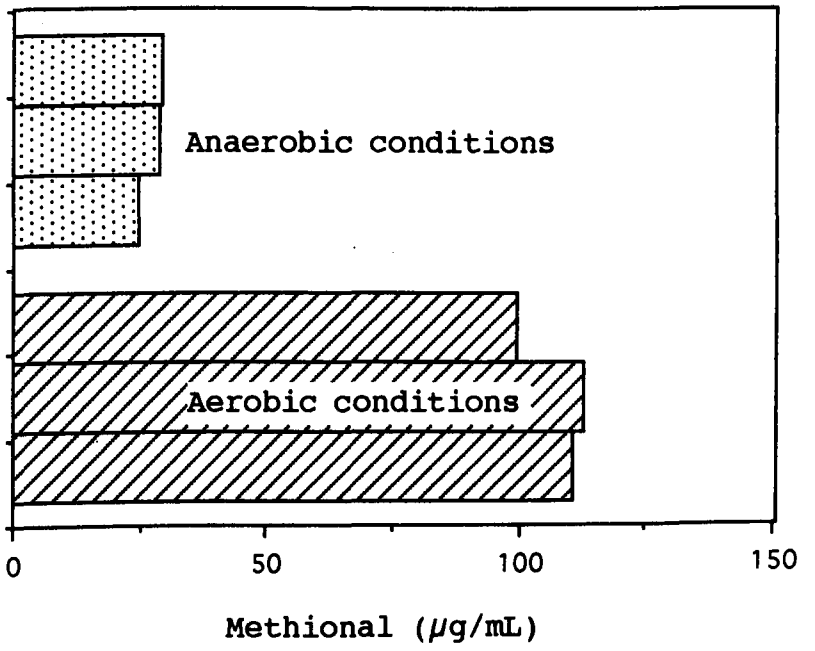

Figure 3.52 Effect of aerobic and anaerobic conditions on methional formation from L-methionine without cupric ion in phosphate buffer

conditions, although that effect was greater on methional formation.

3.4.2.3 Effect of Reducing Sugars on the Formation of Sulphur Compounds from L-Methionine

After fermentation, 0.8-1.4 mM of maltotriose exists in fermented wash of malt whisky. Therefore, the effect of reducing sugars, L-glucose and maltotriose, on the formation of sulphur compounds from L-methionine was investigated.

D-Glucose, maltotriose (Sigma) or no reducing sugar was added to the reaction mixtures with CuAc2 (section 2.3.5). The reaction mixture was heated (section 2.3.6). DMS, DMDS, DMTS and methional concentrations were measured (sections 2.1.2.1 and 2.1.3). The results are shown in Figures 3.53, 3.54 and 3.55.

DMS was not detected due to the overlapping of peaks by another compound. Without reducing sugar, DMDS level in reaction mixture under anaerobic conditions was higher than under aerobic condition. With both D-glucose and maltotriose, DMDS level were higher than without reducing sugar. Under aerobic condition, DMDS levels in reaction mixture with reducing sugars were higher than under anaerobic conditions. There was no effect of reducing sugar on DMTS formation either under aerobic or anaerobic conditions. Under aerobic condition, DMTS level in reaction mixtures without reducing sugar or with D-glucose seemed to be higher than under anaerobic conditions. There was no difference between under aerobic and anaerobic conditions due to a large dispersion. However, with maltotriose, DMTS level in the reaction mixture under aerobic condition was

higher than under anaerobic conditions. With both D-glucose and maltotriose, there were no differences in methional levels in the reaction mixtures between aerobic and anaerobic conditions. Conversely, methional level in the reaction mixture under anaerobic conditions was higher than under aerobic condition. However, with both D-glucose and maltotriose, methional levels in the reaction mixtures either aerobic or anaerobic conditions were higher than without reducing sugar. Under anaerobic conditions, there was no difference between D-glucose and maltotriose on the formation of sulphur compounds from L-methionine. Therefore, D-glucose was used in further experiment.

Cupric ion concentrations of the reaction mixtures before and after reaction were measured (section 2.2). The free cupric ion concentration before reaction was the same

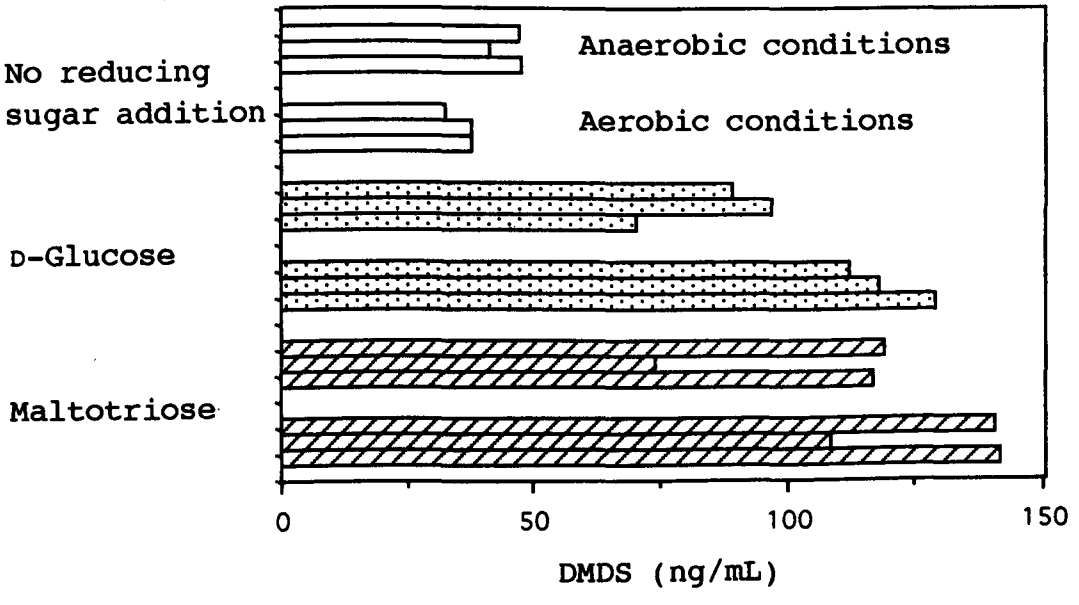

Figure 3.53 Effect of aerobic and anaerobic conditions and reducing sugar on DMDS formation from L-methionine with $CuAc_2$ in phosphate buffer

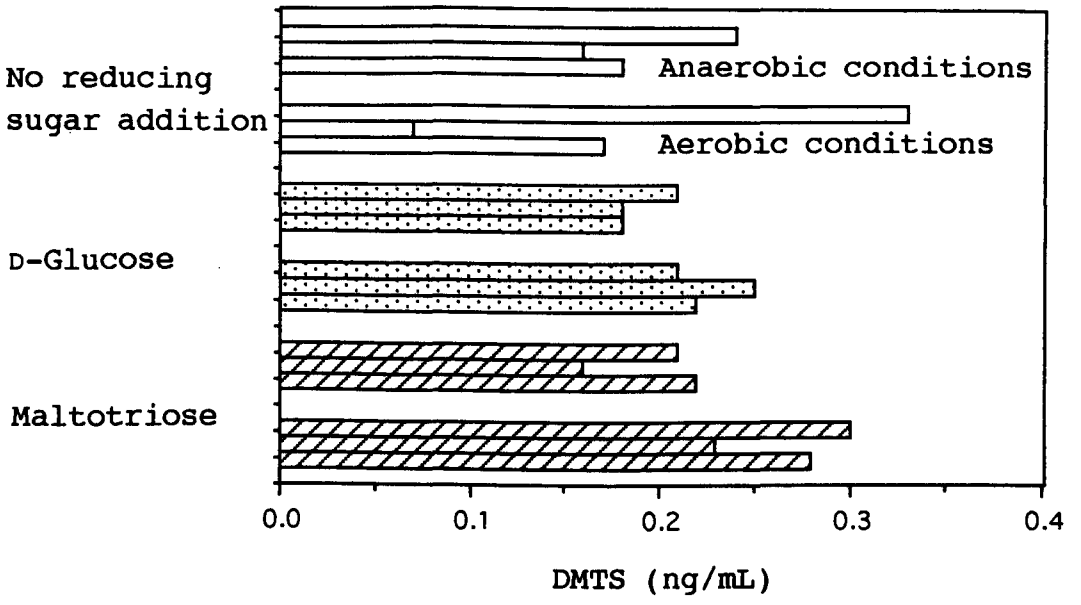

Figure 3.54 Effect of aerobic and anaerobic conditions and reducing sugar on DMTS formation from L-methionine with $CuAc_2$ in phosphate buffer

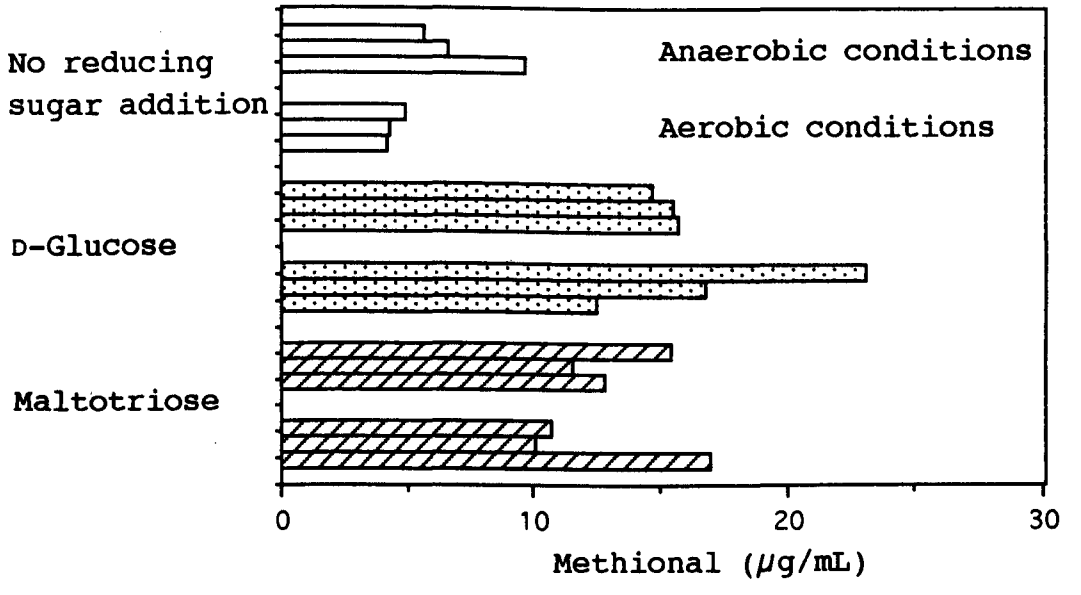

Figure 3.55 Effect of aerobic and anaerobic conditions and reducing sugar on methional formation from L-methionine with $CuAc_2$ in phosphate buffer

as the added amount. There was no change of free cupric ion level in the reaction mixture between before and after the reaction.

3.4.3 Thermal Reaction of L-Methionine

3.4.3.1 Effect of Reaction Time on the Formation of Sulphur Compounds from L-Methionine

In the malt whisky industry, a wash distillation appears to be an anaerobic process. Therefore, the effect of the reaction time on the formation of sulphur compounds from L-methionine under anaerobic conditions was evaluated using phosphate buffer with or without cupric ion.

D-Glucose were added to the reaction mixtures as reducing sugar with $CuAc_2$ (section 2.3.5). The reaction mixture was heated for up to 5 h (section 2.3.6). DMS, DMDS, DMTS and methional concentrations were measured (sections 2.1.2.1 and 2.1.3). The results are shown in Figures 3.56, 3.57 and 3.58.

DMS was not confirmed due to the overlapping of peaks by another compound. With an increase in reaction time, the DMDS level increased in the reaction mixture without $CuAc_2$. Conversely, with $CuAc_2$, DMDS level decreased. DMTS was not detected in any of the reaction mixtures without cupric ion. With cupric ion, DMTS increased with an increase in reaction time. With an increase in reaction time, methional level decreased in the reaction mixture with cupric ion. However, methional level increased a little without cupric ion.

The cupric ion level did not change during reaction of 1-5 h.

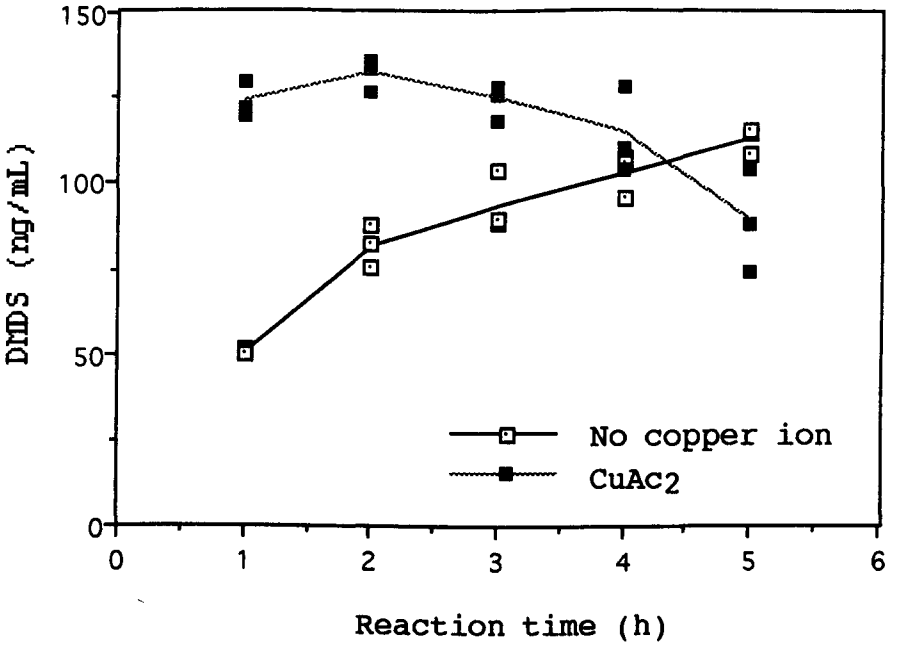

Figure 3.56 Effect of reaction time on DMDS formation from L-methionine with and without cupric ion in phosphate buffer under anaerobic conditions

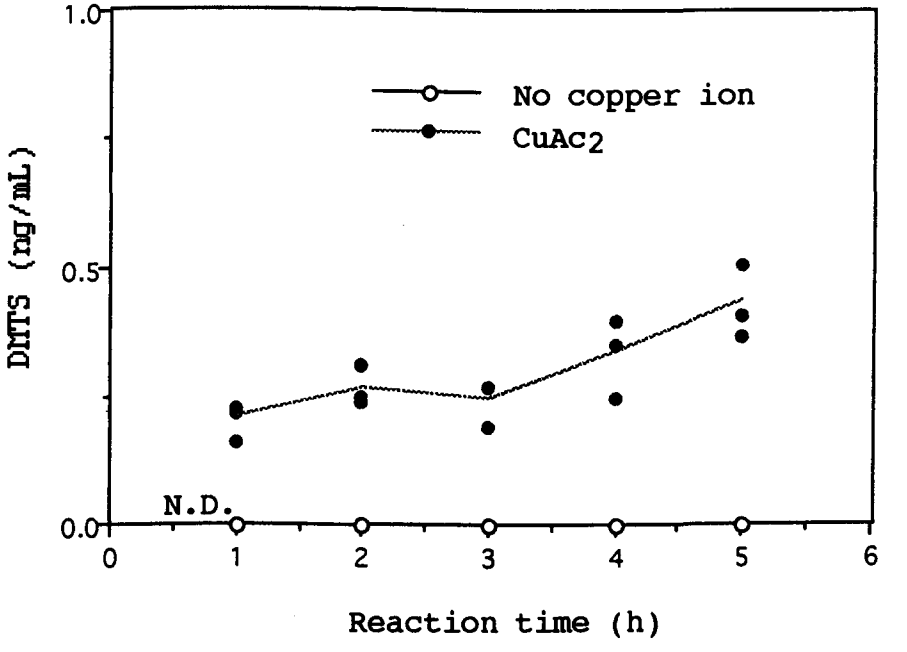

Figure 3.57 Effect of reaction time on the formation of DMTS from L-methionine with and without cupric ion in phosphate buffer under anaerobic conditions

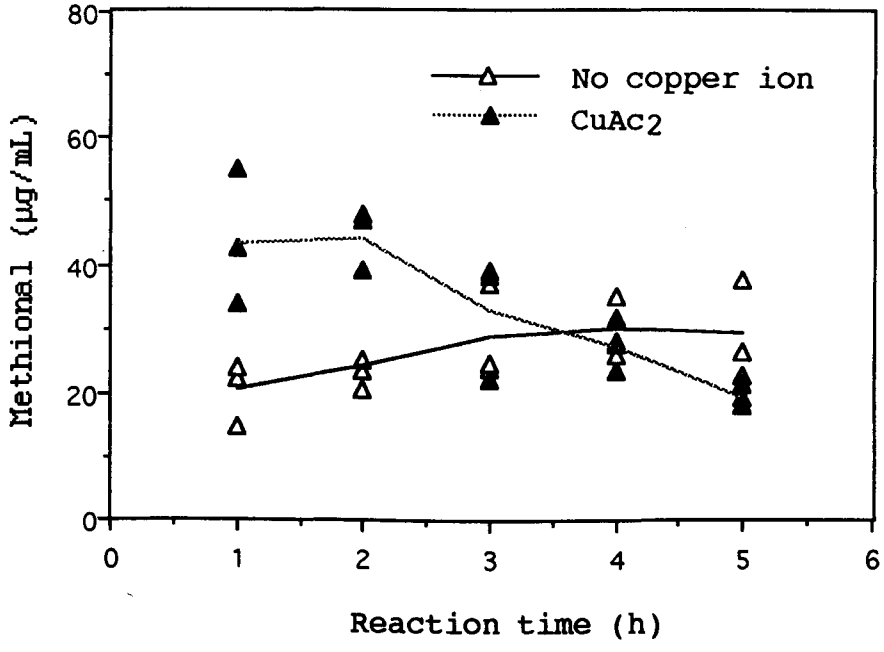

Figure 3.58 Effect of reaction time on the formation of methional from L-methionine with and without cupric ion in phosphate buffer under anaerobic conditions

3.4.3.2 Effect of pH on the Formation of Sulphur Compounds from L-Methionine with and without Cupric Ion

As the quantity of sulphur containing compounds increased with an increase of pH, the effect of pH on the formation of sulphur compounds from L-methionine was investigated with phosphate buffer.

D-Glucose was added to the reaction mixture with or without $CuAc_2$ (section 2.3.5) The pH of solutions were adjusted to 4.0, 5.0, 6.0 and 7.0 by the addition of L-lactic acid. The reaction mixture was heated (section 2.3.6). DMS, DMDS, DMTS and methional concentrations were measured (sections 2.1.2.1 and 2.1.3). Figures 3.59, 3.60 and 3.61

show the results without cupric ion and Figures 3.64, 3.65 and 3.66 show those with cupric ion.

DMS was not detected due to the overlapping of peaks by another compound. With an increase in pH, DMDS level decreased in the reaction mixture with cupric ion. Without cupric ion, DMDS formation from L-methionine was minimal at low pH, the same as the results from methional as described in section 3.3.1.1. DMDS level at pH 7.0 was about twice as much as at pH 4.0. Cupric ion increased the DMDS formation about twice at higher pH. DMDS concentration at pH 7.0 was about 7 times as much as at pH 4.0. As same as DMDS formation, DMTS level from L-methionine was minimal at low pH. Cupric ion did affect the DMTS formation especially at high pH in spite of no effect of cupric ion on the DMTS formation from methional. Both with and without cupric ion, methional level in the reaction mixture was maximal at pH between 5.0 and 6.0, although the levels of methional without cupric ion at pH 5.0 and 6.0 were higher than with cupric ion.

The cupric ion concentrations of the reaction mixtures before and after reaction were measured as described in section 2.2. The results are shown in Figure 3.62.

There was no change in the free cupric ion level in the reaction mixture before and after the reaction. The free cupric ion concentration in the reaction mixture was the same as the added amount between pH 3.0 and 4.0. With an increase in pH, the free cupric ion level in the reaction mixture decreased slightly, although free cupric ion level was about 3 μg/mL over pH 7.0 as described in section 3.3.1.2.

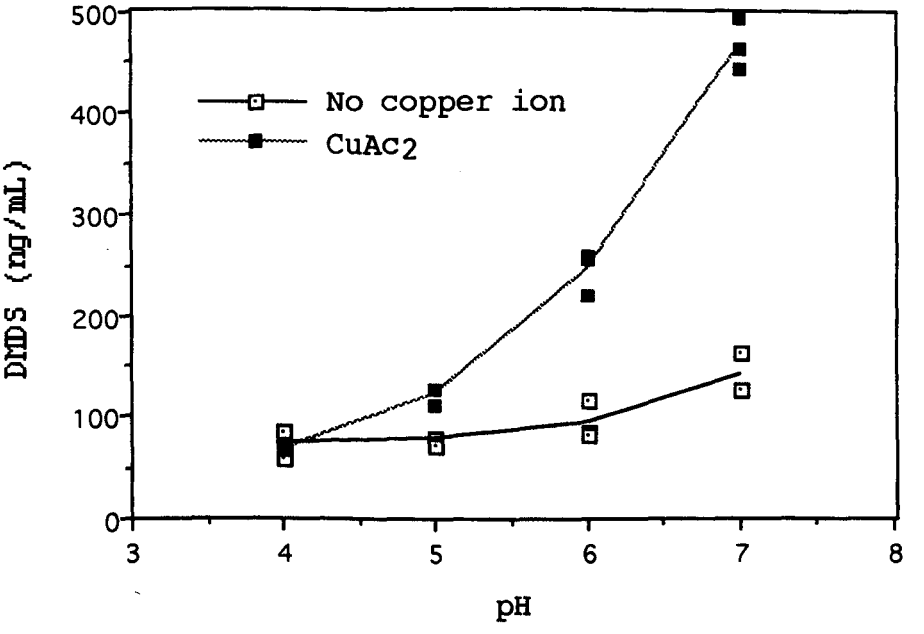

Figure 3.59 Effect of pH on DMDS formation from L-methionine with and without cupric ion in phosphate buffer under anaerobic conditions

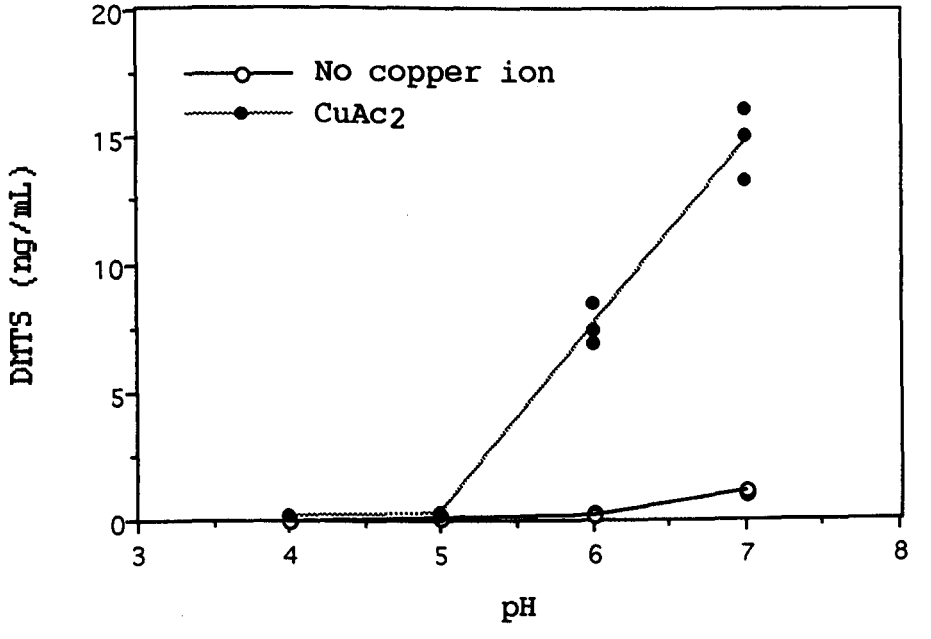

Figure 3.60 Effect of pH on DMTS formation from L-methionine with and without cupric ion in phosphate buffer under anaerobic conditions

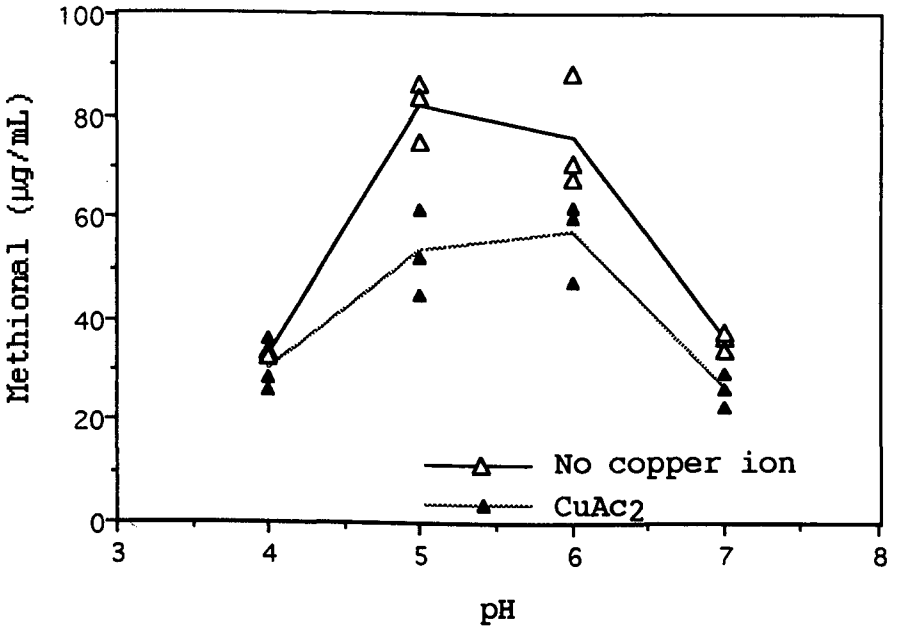

Figure 3.61 Effect of pH on methional formation from L-methionine with and without cupric ion in phosphate buffer under anaerobic conditions

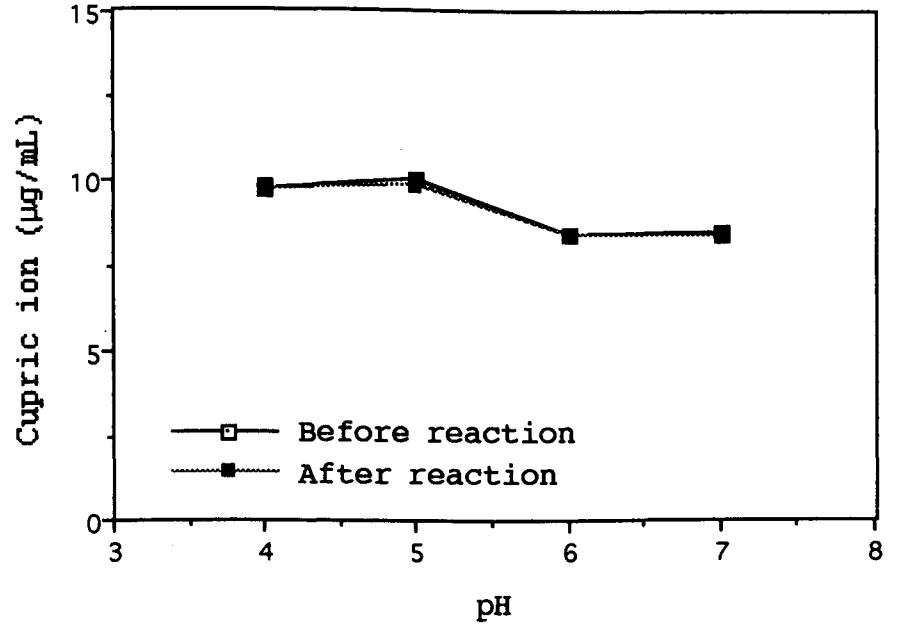

Figure 3.62 Cupric ion concentration of reaction mixture before and after reaction in phosphate buffer

3.4.3.3 Effect of H_2S on the Formation of Sulphur Compounds from L-Methionine and D-Glucose with Cupric Ion

H_2S is known to be needed to provide a sulphur element in the formation of DMTS from methanethiol. Therefore, the effect of H_2S on the formation of sulphur compounds from L-methionine was evaluated using phosphate buffer with D-glucose and cupric ion.

D-glucose were added to reaction mixtures with $CuAc_2$ (section 2.3.5). H_2S was prepared by an acidification of sodium sulphide nonahydrate (Sigma) (Chin and Lindsay, 1994a). One drop of hydrochloric acid was added to 50 mL ethanol solution, 7% (v/v), containing 0.704mg sodium sulphide nonahydrate. This H_2S solution was diluted to 5, 10, 20 μg/mL of H_2S solution. The pH of the solutions were adjusted to 4.5. The reaction mixture was heated (section 2.3.6). DMS, DMDS, DMTS and methional concentrations in the mixtures were measured (sections 2.1.2.1 and 2.1.3). The results are shown in Figures 3.63, 3.64 and 3.65.

DMS was not detected due to the overlapping of peaks by another compound. There was no effect of H_2S on the formations of DMDS and methional from L-methionine with D-glucose and cupric ion. H_2S greatly increased DMTS formation. DMTS level in reaction mixture with 10 ng/mL H_2S was 5 times as much as without H_2S. However, the formation of DMTS did not increase with concentrations over 10 ng/mL H_2S.

Cupric ion concentrations of the reaction mixtures before and after reaction were measured as described in section 2.2.

There was no change of free cupric ion level in the reaction mixture between before and after the reaction. The free cupric ion concentration in the reaction mixture was the same as the added amount between pH 3.0 and 4.0. With an increase in pH, the free cupric ion level in the reaction mixture decreased slightly, although free cupric ion level was about 3 μg/mL over pH 7.0 as described in section 3.3.1.2.

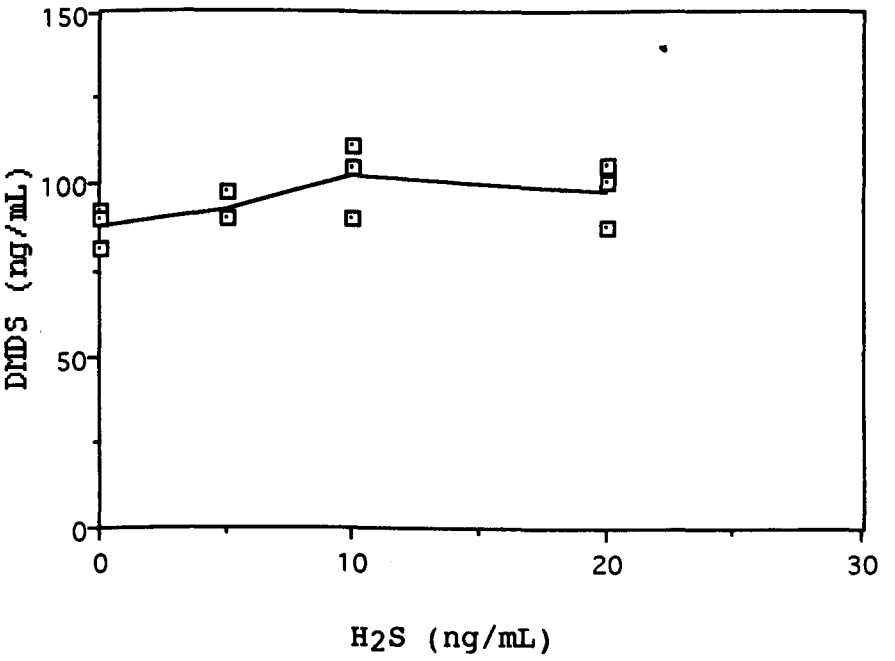

Figure 3.63 Effect of H_2S on DMDS formation from L-methionine with cupric ion in phosphate buffer under anaerobic conditions

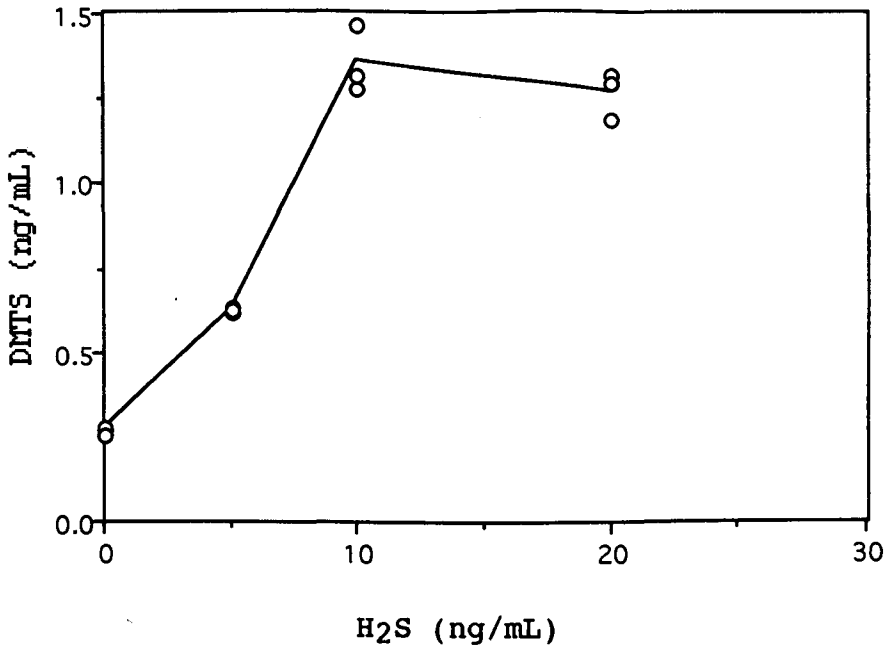

Figure 3.64 Effect of H_2S on DMTS formation from L-methionine with cupric ion in phosphate buffer under anaerobic conditions

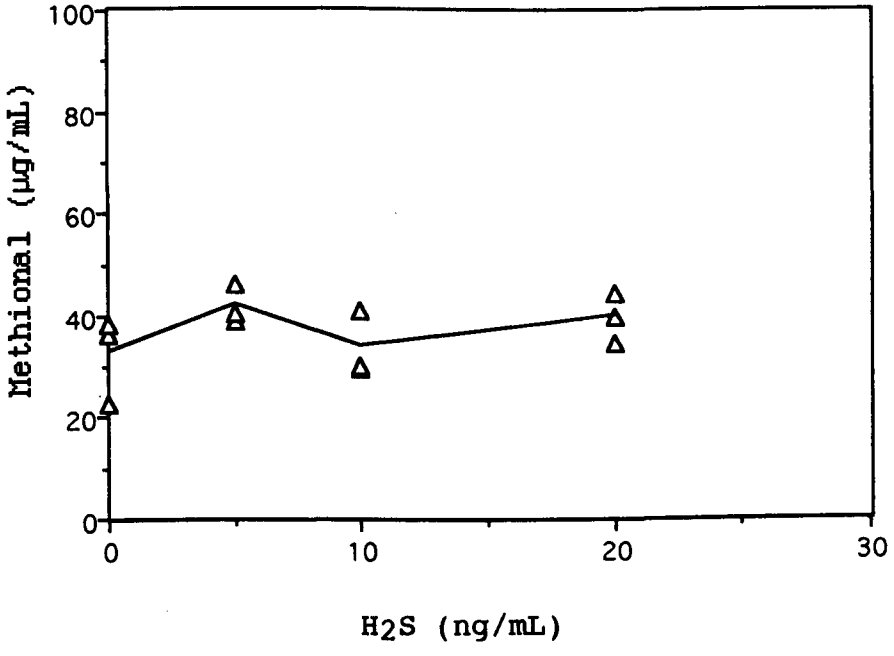

Figure 3.65 Effect of H_2S on methional formation from L-methionine with cupric ion in phosphate buffer under anaerobic conditions

3.4.3.4 Effect of L-Cysteine on the Formation of Sulphur Compounds from L-Methionine and D-Glucose with Cupric Ion

H_2S is known to be needed to provide a sulphur element in the formation of DMTS. H_2S is also known to be formed during the Strecker degradation of cysteine with a diketone. Therefore, the effect of L-cysteine on the formation of sulphur compounds from L-methionine was evaluated.

D-Glucose were added to reaction mixtures as reducing sugar with $CuAc_2$ (section 2.3.5). L-cysteine hydrochloride (BDH), 0, 50 and 100 μM, were added to the reaction mixtures. The reaction mixture was heated (section 2.3.6). DMS, DMDS, DMTS and methional concentrations were measured (sections 2.1.2.1 and 2.1.3). The results are shown in Figures 3.66, 3.67 and 3.68.

DMS was not detected due to the overlapping of peaks by another compound. There was no effect of L-cysteine on the DMDS, DMTS and methional formations from L-methionine with D-glucose and cupric ion.

Cupric ion concentrations of the reaction mixtures before and after reaction were measured (section 2.2).

The free cupric ion concentration in the reaction mixture before the reaction was the same as the added amount. There was no change of free cupric ion level in the reaction mixture between before and after the reaction.

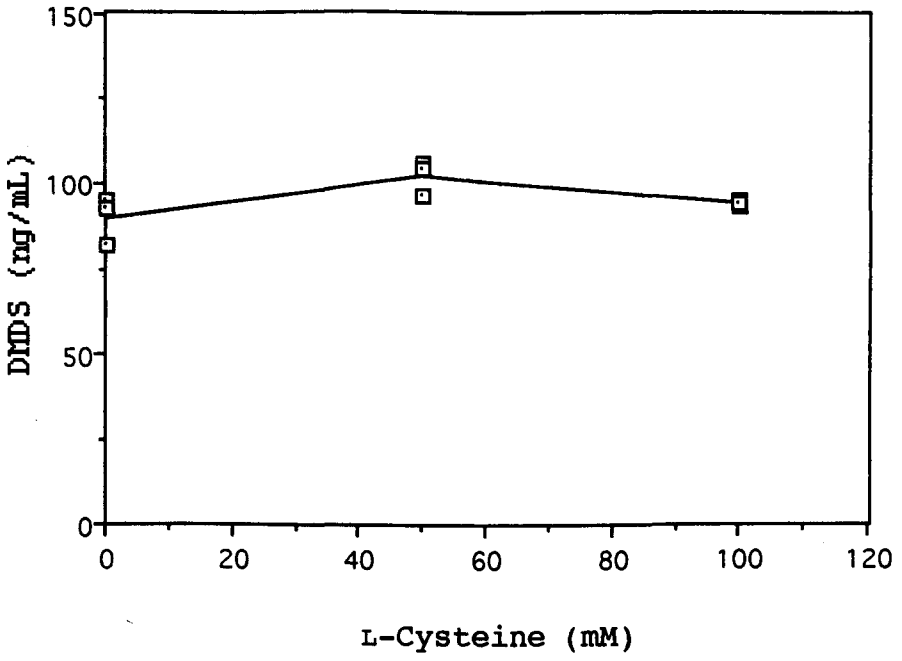

Figure 3.66 Effect of L-cysteine on DMDS formation from L-methionine with cupric ion in phosphate buffer under anaerobic conditions

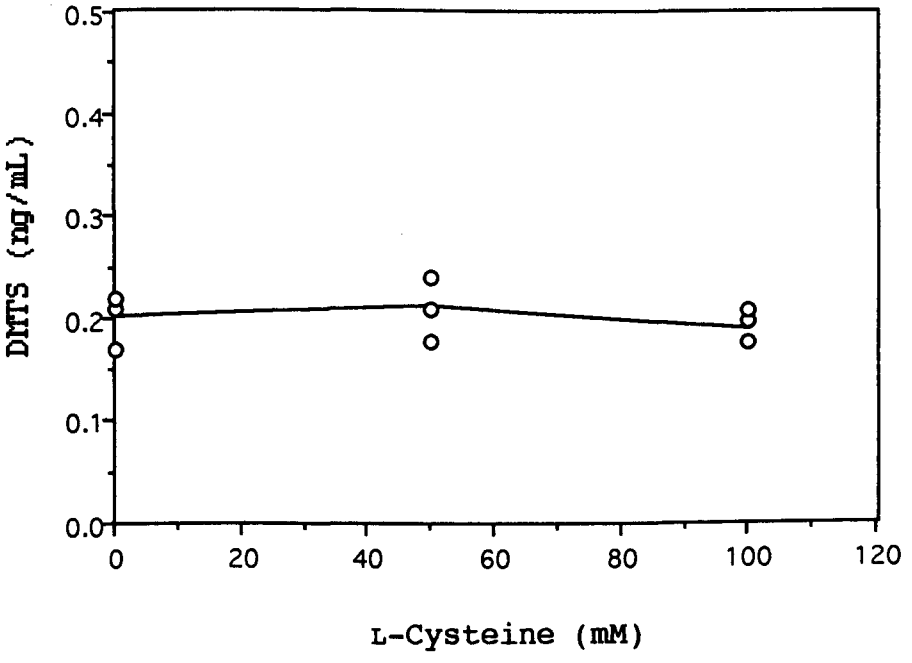

Figure 3.67 Effect of L-cysteine on DMTS formation from L-methionine with cupric ion in phosphate buffer under anaerobic conditions

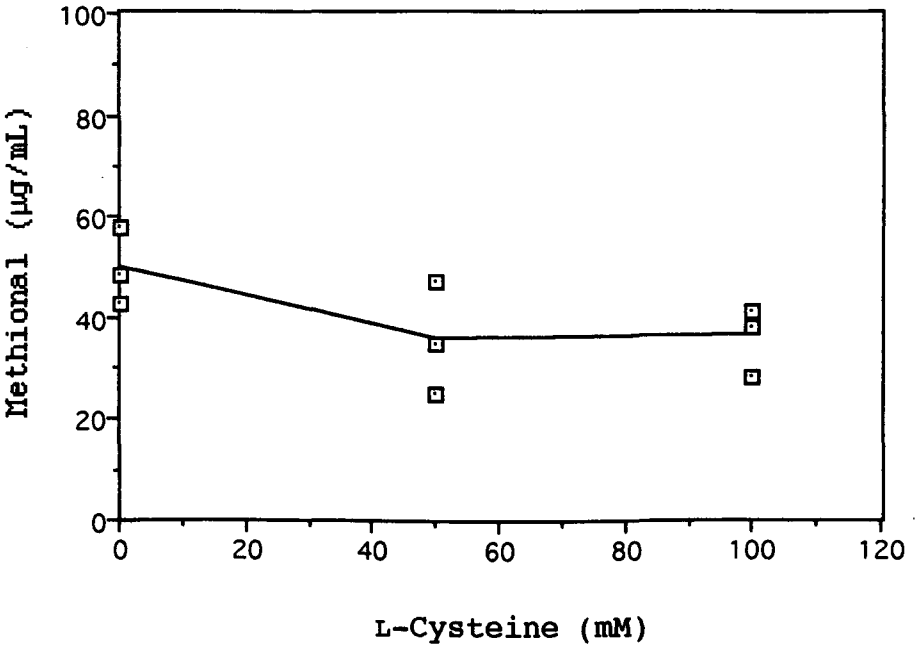

Figure 3.68 Effect of L-cysteine on methional formation from L-methionine with cupric ion in phosphate buffer under anaerobic conditions

3.5 DMTS Formation from DMDS

To confirm the production of DMTS and DMS by the disproportionation of DMDS, the model distillation was examined using the DMDS ethanol solution under the simulated conditions of spirit (second) distillation.

3.5.1 Using Model Distillation System

A 300 mL, 25% (v/v), ethanol solution, containing 354 ng/mL of DMDS, was distilled using the model distillation system (section 2.3.2). The reaction times were within 30 ± 2 min. The volume of collected distillate was 165 mL. DMS and DMDS concentrations in the distillates were measured as described in section 2.1.2.1 and DMTS concentration was measured as described in section 2.1.3.

DMDS was detected at a concentration of 494 ng/mL; SD 8.0 ng/mL. DMS and DMTS were not detected in any of the reaction mixtures. The recovery ratios of DMDS were 76-78% in consideration of the liquid concentration by distillation.

3.5.2 Effect of Copper Salts

The effect of copper salts on the DMTS and DMS formation by the disproportionation of DMDS was evaluated using the DMDS ethanol solution under the simulated condition of spirit (second) distillation. Three cuprous or cupric salts, Cu_2S, CuS and $Cu(CH_3COO)_2 \cdot H_2O$, equivalent to 50 μg/mL (785 μM) Cu(I) or Cu(II), were added to 300 mL ethanol solution, 25% (v/v), containing 354 ng/mL of DMDS. Each reaction mixture was distilled using the model distillation

system (section 2.3.2). The reaction times were within 30 ± 2 min. The volume of collected distillate was 165 mL. DMS and DMDS concentrations in the distillates were measured as described in section 2.1.2.1 and DMTS concentration was measured as described in section 2.1.3. The results are shown in Table 3.12.

DMS and DMTS were not detected in any of the reaction mixtures. The recovery ratios of DMDS were 72-80% in consideration of the liquid concentration by distillation.

Table 3.12 DMDS concentration (ng/mL) in the reaction mixture

Run No.	1	2
Cu_2S	466.3	470.1
CuS	467.0	474.8
$Cu(CH_3COO)_2 \cdot H_2O$	461.8	516.0

4. DISCUSSION

Due to their importance as flavour compounds, this project focused on the formation and reactions of sulphur compounds, especially DMDS and DMTS, during distillation of fermented malt whisky wash. Little information is available on the formation of DMDS and DMTS during whisky distillation (section 1.3.4). Although many model systems have been published in flavour research (Table 1.6), none have simulated the conditions, pH 3-5, temperature max 100 °C, of malt whisky distillation.

The aim of this project was therefore to investigate the mechanism of formation of DMDS and DMTS. The level of DMTS is very important in malt whisky spirits because of its very low flavour threshold (15 ng/mL) and very slow decline during maturation.

This project is divided mainly into two parts:

(a) the formation of sulphur compounds from methional,

(b) the formation of sulphur compounds from methionine.

The following are the main factors investigated in both parts:

- Reaction time
- pH of reaction mixtures
- Copper salts
- Aerobic and anaerobic conditions

4.1 Thermal Decomposition of Methional

To gain a better understanding of the mechanism of formation of DMDS and DMTS in whisky spirit and the factors which affect these formations, a methional model system was investigated. It was observed that several factors, time of

heating, copper salts, amount of copper salt, anions and pH affected the formation of sulphur compounds from methional. These factors are discussed in the next sections.

Both DMDS and DMTS were identified in the products from the methional model system, confirming the theory of Yang *et al.* (1967) that DMDS was formed from methionine via methional.

4.1.1 Effect of Duration of Distillation Experiment

The amount of volatile sulphur compounds formed, such as DMDS and DMTS, increases with heating time (Figures 3.14 and 3.15). In Scotch malt whisky distilleries the duration of wash still distillation is normally 4-6 h. It was not possible to extend the reaction time of a small-scale distillation to more than 1 h, hence the trial of the decomposition of methional by the combination of model distillation and reflux for 6 h (section 3.2.6). Of course in malt whisky distilleries the temperature of the wash increases gradually during distillation due to the decrease of ethanol concentration, whereas the temperature of the refluxed reaction mixture remained constant, and lower than in wash distillation.

DMDS concentration in the distillate of the 6 h reflux distillation was about 3 times that in simple distillation for 49 min, but this only occurred when cupric was added (Figures 3.14 and 3.32). Because of the short duration in experimental distillation, it is expected that in a commercial distillation the level of DMDS in low wines is higher than model distillation.

However, DMTS concentration was reduced (Figures 3.15 and 3.33). Thus, the formation of DMDS from methional can occur more easily than DMTS.

With no addition of copper salts, both DMDS and DMTS concentrations in the refluxed distillation sample were about half those of simple distillation. Either DMDS and DMTS appeared to change to another sulphur compound during the prolonged distillation.

In the refluxed distillation, DMDS concentration with copper salts was about 100 times that without copper salts (Figure 3.32) indicating that cupric ion in the reaction solution catalysed the formation of DMDS from methional. This result is consistent with the conclusion of Masuda and Nishimura (1981). When wash was distilled in glass, the DMDS concentration in low wines was about one tenth that in a copper still. On the other hand copper metal is understood to remove or modify the unpleasant odour caused by volatile sulphur compounds (Tanner, 1969; Hardy and Brown, 1989; Whitby, 1992; Piggott and Conner, 1995).

It must be remembered that the experimental distillation system was glass, copper was absent from the head of still and condenser, although the still neck, lyne arm and condenser are constructed of copper in malt whisky distilleries. The level of DMS in the distillate was lower in a copper pot still than in a glass still (Swan and Burtles, 1978), and DMDS and DMTS may be reduced in the same way.

Therefore, copper has two opposing functions for the sulphur compounds. One is the catalysis of the formation of sulphur compounds: copper or cupric ion in the reaction

solution can catalyse the formation of DMDS from precursor. The other is the well-known elimination function of sulphur compounds, such as H_2S and DMS.

4.1.2 Effect of Cupric Ion Concentration

In a commercial distillery, copper concentration increases gradually during distillation (Hardy and Brown, 1989). The low pH of the wash dissolves copper and the acid liquid condensed in the neck and the head of the still dissolves copper and then returns to the wash. On a laboratory scale, it is very difficult to control the amount of copper which dissolves from copper plate, therefore, soluble copper salts were added in various known amounts.

Formation of both DMDS and DMTS increased with addition of cupric ion up to 50 μg/mL (Figures 3.16 and 3.17). However, the concentration of dissolved copper in pot ale, the waste liquid from wash stills, in the Irish and Scotch malt whisky industries, has been reported to reach 15 μg/mL (Quinn *et al.*, 1981). Therefore, to achieve the obvious effect of copper ion on the formation of sulphur compounds from methional, 10 μg/mL of copper ion was added to a reaction solution by consideration the liquid volume reduction due to distillation. The presence of cupric ion in the reaction mixture at the begining of the experimental distillation is not the case in a commercial distillation, in which copper ion concentration is zero at the start and gradually increases throughout distillation.

4.1.3 Effect of Copper Salts and Other Compounds

As discussed in section 4.1.2, cupric ion increased the DMDS and DMTS concentration in the distillate from methional. $CuAc_2$ was used as cupric ion. However, it is not clear that either only cupric ion or other compounds can have a such effect. Therefore, seven different copper salts (Cu_2S, CuS, CuCl, $CuCl_2$, CuO, $CuSO_4$ and $CuAc_2$) were investigated.

It was hypothesised that copper ion which dissolve or is free from the copper complex with chelate, such as EDTA, increased the formation of DMDS and DMTS from methional. Nevertheless, conflicting observations were obtained (Figures 3.18, 3.19 and 3.20):

- $CuSO_4$ and $CuCl_2$ did not increase the formation of DMDS and DMTS, although these copper salts dissolved as a cupric ion.
- CuS and Cu_2S, which have low solubilities, increased the formation of DMDS and DMTS from methional, although cupric ion concentrations in these reaction mixtures were nearly zero.

Matheson (1965) reported that cupric ion associates with sulphate ion in aqueous solution. The cupric ion forms cationic species with chloride in aqueous solution (Bailar *et al.*, 1973). Therefore, it appears that the cupric ion is surrounded by sulphate or chloride ion and does not make contact with the methional molecule. Thus the addition of $CuSO_4$ or $CuCl_2$ does not increase the formation of DMDS and DMTS from methional. Conversely, the copper atom of CuS and Cu_2S appears to be able to react with methional, although the copper atom associates strongly with the sulphur atom. Hence, it appears to be important whether the copper atom,

either copper salt or copper ion, can directly attack the precursor molecule or not.

4.1.4 Effect of Ethylendiamintetraacetic Acid

It is well-known that EDTA is a chelating agent and makes a stable complex ion with equimolar cupric ion. EDTA has 6 ligands each of which makes a coordinate bond with cupric ion. Thus, cupric ion is surrounded by EDTA (Figure 4.1). As discussed in section 4.1.3, it was hypothesised that cupric ion which is surrounded by anions cannot increase the formation of sulphur compounds. Therefore, inhibition by EDTA was investigated.

Addition of $CuAc_2$ to the reaction mixture increased the formation of both DMDS and DMTS from methional, and it seems that EDTA inhibited this effect (Figures 3.24 and 3.25). It appears that only free cupric ion can affect the formation of DMDS and DMTS from methional. However, these reaction mixtures were not buffered and EDTA is a HCl salt. Thus, the pH of the reaction mixture with addition of $CuAc_2$ plus EDTA was lower than other mixtures. As described later, in section 4.1.6, at lower pH, the formation of DMDS and DMTS from methional decreased. Therefore, with addition of $CuAc_2$ plus EDTA, the levels of DMDS and DMTS were lower than that with addition of $CuAc_2$ due to both EDTA and the reduction of pH.

4.1.5 Effect of Aerobic and Anaerobic Conditions

Schutte (1974) suggested that oxygen might participate in the formation of DMDS from methanethiol, which is formed by decomposition of methionine. Therefore, the effect of

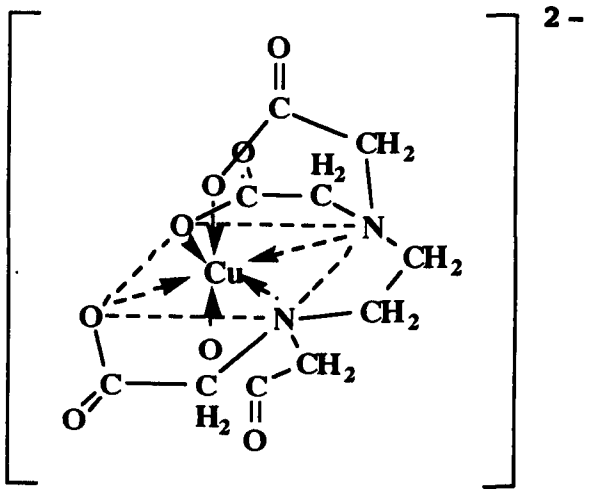

Figure 4.1 Complex ion of cupric ion and EDTA

aerobic and anaerobic conditions on the formation of sulphur compounds from methional was investigated. The formation of DMDS and DMTS from methional was unaffected by aerobic or anaerobic conditions (Figures 3.28 and 3.29). Therefore, it is clear that oxygen does not participate in the formation of DMDS from methional. Moreover, the physical effect of bubbling did not decrease the DMDS and DMTS concentration, although DMDS and DMTS are highly volatile.

4.1.6 Effect of pH

Generally, the quantity of sulphur containing compounds increased with pH of the reaction mixtures. Therefore, the effect of pH on the formation of sulphur compounds from methional was investigated with phosphate and sulphate solutions.

With an increase in pH, both DMDS and DMTS concentration increased in the distillates in phosphate buffer with or without addition of $CuAc_2$ (Figures 3.34, 3.35 and 3.36). $CuAc_2$ increased greatly the formation of DMDS from methional

especially at high pH, although $CuAc_2$ did not increase the formation of DMTS.

The similar results, which the formation of DMDS was increased at higher pH, were reported from methionine model systems (Mottram and Leseigneur, 1990; Chan and Reineccius, 1994). However, they did not use any copper salt. In the model system of Mottram and Leseigneur, the methionine solutions were reacted at 140 °C for 1 h at pH 4.5, 5.5 and 6.5. Thus, the formation of DMDS from methionine may occur through a different reaction pathway due to such a high temperature. Chan and Reineccius heated the methionine with glucose at 95 °C for 100 min. Although the temperature was constant, the conditions were otherwise similar to distillation conditions. However, they reacted at pH values only between 6 and 8. Their results did not give us enough information, as the pH of malt whisky fermented wash is 3.7-4.5 (MacKenzie and Kenny, 1965; Barbour and Priest, 1988; Makanjuola *et al.*, 1992).

With phosphate buffer, between 3.0 and 5.0 the formation of DMDS was increased by the addition of CuAc2. Therefore, the dissolved copper from the still during wash distillation must increase the level of DMDS in low wines.

The formation of DMDS and DMTS from methional was maximal at pH 5.0 in sulphate solution without copper salt (Figures 3.40 and 3.42). In general, the quantity of sulphur containing compounds increased with an increase of pH (Chan and Reineccius, 1994), Therefore, it appears that the addition of sulphate ion suppressed the formation of DMDS and DMTS between pH 6.0 and 8.0 without copper salt. At high pH, the addition of $CuAc_2$ increased the formation of both

DMDS and DMTS in both sulphate and phosphate buffer (Figures 3.41 and 3.42) However, the formation of DMDS and DMTS decreased between pH 3.0 and 5.0 in sulphate solution. Thus, the variety and the quantity of anions in the fermented wash appear to affect the formation of DMDS and DMTS.

During a malt whisky fermentation rapid growth of lactic acid bacteria may occur, resulting in the pH of the wash falling to 3.7-4.1 by the end of fermentation (MacKenzie and Kenny, 1965; Barbour and Priest, 1988; Makanjuola *et al.*, 1992). As described above, the formation of DMDS and DMTS from methional depressed at low pH. Hence, the infection of lactic acid bacteria appears to affect the level of DMDS in the low wines.

4.1.7 Formation Mechanism of DMDS and DMTS from Methional

In both phosphate and sulphate solutions, although the absolute quantities are different, the formation of DMTS with and without addition of $CuAc_2$ showed the same trends as the results for the formation of DMDS. Therefore, the pathways of DMDS and DMTS from methional appear to have the same steps. Chin and Lindsay (1993) reported that DMDS and DMTS had similar production patterns in disrupted cabbage tissues at 30 °C and they suggested that these two sulphur compounds might have a common precursor. Ballance (1961) identified methanethiol from the decomposition of methional with ninhydrin.

Chin and Lindsay (1994a) reported that the presence of ascorbate plus Fe(III) or Cu(II) resulted in a rapid formation of DMDS and DMTS from methanethiol and H_2S at 30 °C. Although the temperature of their experiment was only at

30 °C, this reaction must occur easily at about 100 °C. Therefore, the formation pathway of DMDS and DMTS from methional can be explained into two steps (Figure 4.2).

First step is the formation of methanethiol from methional. This reaction appears to be controlled by pH of solution and anion. Second step is the formation of DMDS and DMTS from methanethiol. This reaction can occur at 30 °C (Chin and Lindsay, 1994a). Therefore, first step appears to be the rate-determining step.

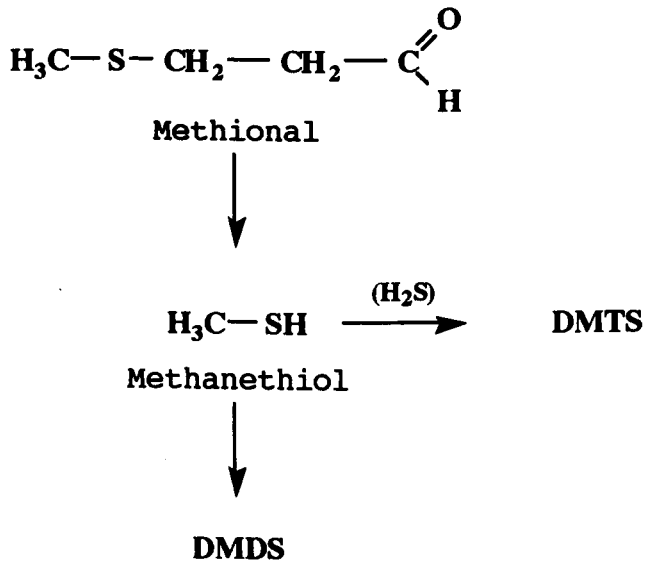

Figure 4.2 The formation reaction of DMDS and DMTS from methional (Ballance, 1961; Chin and Lindsay, 1994a)

4.2 Thermal Decomposition of L-Methionine

Yang *et al.* (1967) proposed that DMDS was formed from methionine through methional. Later, many authors (Wainwright, 1971; Schutte and Koenders, 1972; Shankaranarayana *et al.*, 1974; Shankaranarayana, 1982; Vernin and Párkányi, 1982; Hoskin and Dimick, 1984b) suggested the same mechanism.

Three formation mechanisms have been proposed for the formation of DMTS. One is the insertion of a sulphur atom into the DMDS molecule (Reid, 1960; Peppard, 1978).

$$DMDS + S \longrightarrow DMTS$$

Reid's (1960) mechanism referred to the petroleum industry. It is possible that this insertion reaction may occur only under the high temperature conditions of oil refining.

A second possibility is the rearrangement (Schutte, 1974; Hoskin and Dimick, 1984b), although proposed by Schutte without any experimental evidence.

$$2DMDS \longrightarrow DMTS + D\dot{M}S$$

The third is oxidation. Chin & Lindsay (1994a) recently detected DMTS from methanethiol and H_2S in the presence of H_2O_2 or ascorbate plus Fe(III) or Cu(II). They also showed that H_2S was needed to produce DMTS from methanethiol in oxidative formation (Chin and Lindsay, 1994a; 1994b).

4.2.1 Thermal Reaction of L-methionine with H_2O_2 or L-Ascorbic Acid

Lieberman *et al.* (1965) identified DMDS but not DMTS in the decomposition products of methionine with H_2O_2 or L-ascorbic acid and copper catalyst. Therefore, the effect of H_2O_2 and L-ascorbic acid on the formation of sulphur compounds from methionine was investigated. H_2O_2 greatly increased the formation of DMDS and DMTS from L-methionine, although L-ascorbic acid has a slight effect (Figures 3.46 and 3.47). It is known that L-ascorbic acid can produce H_2O_2 (Coultate, 1984). Thus, H_2O_2 can oxidise the L-methionine to

methional, which can then be decomposed to DMDS and DMTS as described in section 4.1. However, there is no such strong oxidising agent in fermented wash.

4.2.2 Arroyo's Experiment

DMDS and DMTS were not detected from the thermal reaction of L-methionine using model distillation system at any pH (section 3.4.1.2). However, Arroyo and Lillard (1970) identified DMS and DMDS from methionine model system with glucose. There are two different conditions between Arroyo and Lilland (1970) and this project's experiments.

One is the method of heating, and reaction time. The reaction mixtures were heated in a water bath at 100 °C for 2 h under their conditions, whereas the reaction mixtures were distilled for 30 min in the model distillation system of this project.

The other difference is the amount of methionine. In this project, about one third of methionine was used compared to their experiment. Therefore, the formation of sulphur compounds from methionine was investigated under the experiment of Arroyo and Lillard, 1 mM methionine, reaction time 2 h. A centrifuge tube with a screw cap was used as a sealed vial to avoid the evaporation of sulphur compounds. Simultaneously, the effects of diketone and isomer were investigated.

With the addition of D-glucose, DMDS and methional were identified in the reaction mixture (Table 3.11). Therefore, DMDS was not detected (section 3.4.1.2), as the amount of L-methionine was too little. D-Glucose is also necessary for the formation of DMDS from methionine. There was no

difference between L-methionine and DL-methionine. Thus, L-methionine was used in a further experiment. A thermal reaction system using a sealed vial was used for the decomposition of L-methionine instead of model distillation to analyse methional concentration correctly in the reaction mixture. Methional can only be partially distilled. Also the reaction time can be changed easily using sealed vial system.

4.2.3 Effect of Aerobic and Anaerobic Conditions

It has been suggested that oxygen participates in the formation of DMDS from methanethiol which is formed from the decomposition of methionine. A strong oxidant, such as H_2O_2, increased the formation of DMDS and DMTS from L-methionine (section 3.4.1.1). Therefore, the effect of aerobic and anaerobic conditions on the formation of sulphur compounds from L-methionine was investigated.

Without copper salts, the levels of both DMDS and methional under aerobic conditions were higher than under the anaerobic conditions (Figures 3.52 and 3.53). Nevertheless, with addition of $CuAc_2$, the levels of DMDS, DMTS and methional were same between aerobic and anaerobic conditions (Figures 3.53, 3.54 and 3.55). Elemental copper and copper salts are used as an oxidation catalyst (Hudlicky, 1990). Hence, due to this oxidation catalysis of $CuAc_2$, it appeared to have no difference in the level of sulphur compounds from L-methionine between aerobic and anaerobic conditions.

Air exists in the wash still before the charging of the wash and a small amount of oxygen may be introduced at this

point. The air in the headspace of the wash still is replaced by foam and carbon dioxide gas released from the wash during early stage of wash distillation. Although a wash distillation may be an anaerobic process in the malt whisky industry, the oxidation of some compounds in fermented wash appears to occur due to copper catalysis during wash distillation.

4.2.4 Effect of Reducing Sugars

Although D-glucose increased the formation of DMDS and methional from methionine without the addition of copper salt (section 3.4.2.1), glucose and maltose do not exist in the wash after fermentation. However, 0.8-1.4 mM of maltotriose exists in fermented wash of malt whisky (Maemura, 1993). Therefore, the effect of reducing sugars, D-glucose and maltotriose, on the formation of sulphur compounds from L-methionine was investigated.

The addition of both D-glucose and maltotriose increased the formation of DMDS and methional from methionine with and without copper salt, although there was no effect of reducing sugar on the formation of DMTS (Table 3.11 and Figures 3.53, 3.54 and 3.55). DMDS was identified from many methionine model system with not only monosaccharide as glucose but also polysaccharide as pectine (Table 1.6). Besides, Ballance (1961) identified DMDS in products of degradation of methionine with ninhydrine which is diketone. Therefore, carbonyl compounds, such as aldehydes, ketones and reducing sugars, are involved in the degradation of α-amino acid (MacLeod, 1994). MacLeod and Ames (1988) proposed the formation mechanism of Strecker aldehyde such as

methional from α-amino acid with dicarbonyl compounds (Figure 4.3).

$CuAc_2$ increased the formation of DMTS from L-methionine, as DMTS could not be detected in the previous experiments (section 4.2.3). This result was consistent with that $CuAc_2$ increased the formation of DMTS from methional at pH 4.0 (Figure 3.36).

4.2.5 Effect of Reaction Time with and without Cupric Acetate

A wash distillation appears to be an anaerobic process. Therefore, the effect of the reaction time on the formation of sulphur compounds from L-methionine under anaerobic conditions was evaluated using phosphate buffer, pH 4.5, with or without cupric ion in sealed vial. Although in malt whisky distilleries, the temperature of wash increased gradually during wash distillation, the temperature of reaction mixtures was constant at 100 °C in this experiment.

With an increase in reaction time, the DMDS level increased in the reaction mixture without $CuAc_2$. After 5 h the formation of DMDS from L-methionine was still occurring slowly. Conversely, with $CuAc_2$, DMDS level decreased (Figure 3.56). In appearance, it seems that the formation reaction of DMDS from L-methionine has finished within 2 h. However, the methional level also decreased with an increase in reaction time (Figure 3.58). Thus the formation reaction of DMDS from methional must occur continuously. DMS in the distillate from a copper pot still was reduced by 70% compared to all-glass distillation (Swan and Burtles, 1978).

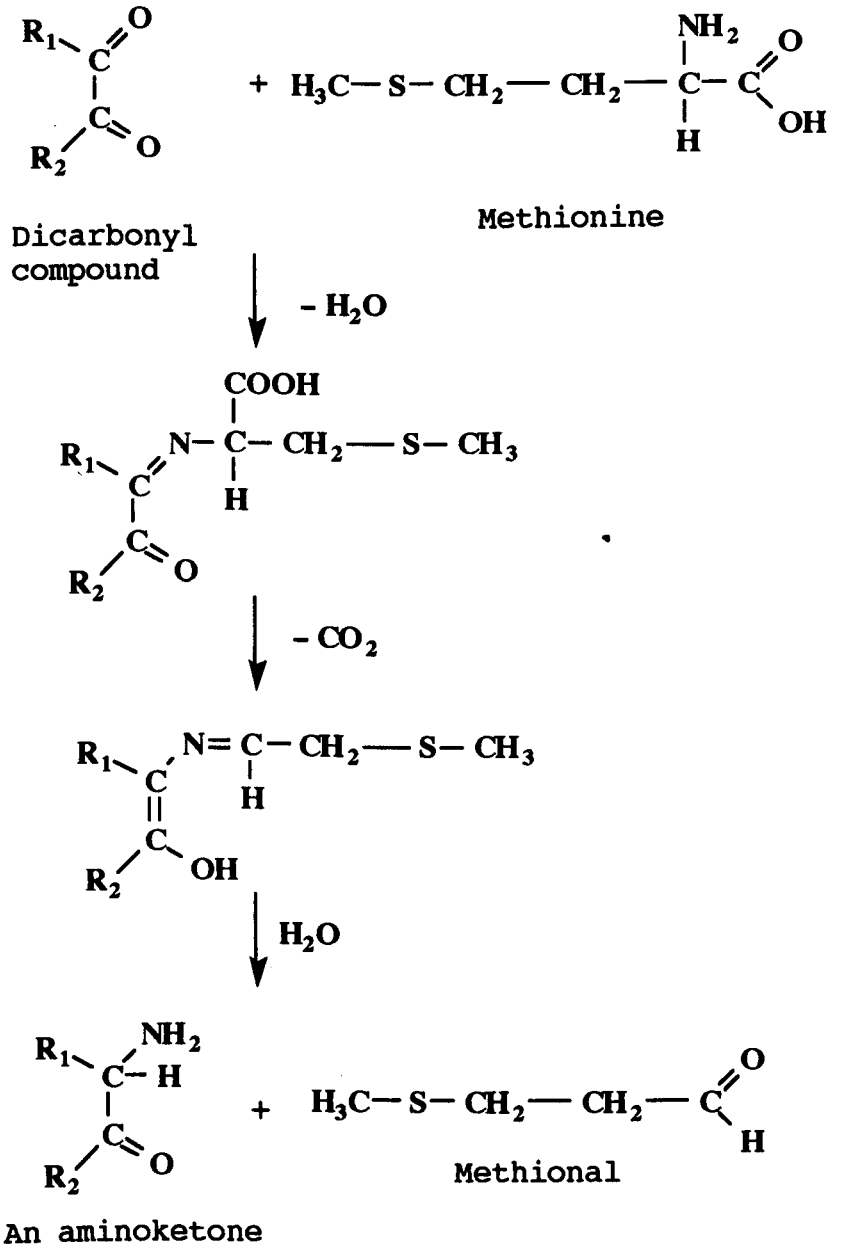

Figure 4.3 The Strecker degradation of methionine with diketone (MacLeod and Ames, 1988)

The recovery ratio of DMTS is only 40% in most cases in spirit still distillation (Beveridge, 1990). In model distillation the recovery ratios of DMDS in the presence of copper salts decreased to the same levels as without copper salts (section 3.5). As a sealed vial was used in this reaction system, the formed DMDS from L-methionine stayed in the solution and could have an opportunity of contact with cupric ion. Therefore, it appears that DMDS formed from L-methionine react with cupric ion to convert to another compound. As DMDS level decreased with an increase in reaction time, this conversion reaction was faster than the formation reaction of DMDS.

DMTS was not detected in any of the reaction mixtures without cupric ion. With cupric ion, DMTS increased with an increase in reaction time (Figure 3.57). Therefore, cupric ion or copper salt plays an important role in the formation of DMTS from L-methionine. Nevertheless, this results was not consistent with that of DMDS. The recovery ratio of DMTS is only 40% in most cases in spirit still distillation (Beveridge, 1990). Thus DMTS can react with copper. During maturation, the level of DMTS declined more slowly than DMS and DMDS (Perry, 1986). Although temperature conditions are different between maturation and this thermal reaction, this lower reactivity of DMTS appears to apply to the thermal reaction conditions.

As the methional level with $CuAc_2$ after 1 h was higher than without $CuAc_2$, it appeared that cupric ion could catalyse the decomposition of L-methionine. No publication is known at present confirming this result under the these experimental conditions.

4.2.6 Effect of pH on the formation of Sulphur Compounds from L-methionine with and without Cupric Acetate

In the same way as for methional, the effect of pH on the formation of sulphur compounds from methionine was investigated with phosphate.

With an increase in pH, DMDS and DMTS concentration increased in the reaction mixtures with and without addition of $CuAc_2$ (Figures 3.59 and 3.60). These results were consistent with the formation of DMDS and DMTS from methional in phosphate buffer (Figures 3.34, 3.35 and 3.36). Therefore, as proposed by Yang (1967), the formation of DMDS and DMTS from methionine. The formation of DMDS and DMTS from methional has been already discussed in section 4.1.6.

Both with and without cupric ion, the methional level in the reaction mixture was maximal at pH between 5.0 and 6.0 (Figure 3.61). As the formation of DMDS and DMTS from L-methionine was increased with an increase in pH, the formation of methional from L-methionine ought to be increased with an increase in pH. However, the methional level decreased at high pH. Thus, the formation reaction of DMDS and DMTS from methional appears to be faster than the formation of methional from L-methionine.

4.2.7 Effect of H_2S

Chin and Lindsay (1994b) reported that H_2S was involved in the formation of DMTS from methanethiol. Therefore, the effect of H_2S on the formation of sulphur compounds from L-methionine was evaluated using phosphate buffer, pH 4.5, with D-glucose and cupric ion.

H_2S greatly increased the formation of DMTS from L-methionine with D-glucose, although there was no such effect of H_2S on the formations DMDS and methional from L-methionine (Figures 3.63, 3.64 and 3.65). As H_2S plays an important role in the formation of DMTS from L-methionine, the formation mechanism of DMTS from methionine appears to be through methanethiol and H_2S is needed to provide a sulphur element in the oxidative formation of DMTS from methanethiol (Chin and Lindsay, 1994b). Hence, the control of the level of H_2S in fermented wash is a key point for the DMTS concentration in low wines.

4.2.8 Effect of L-Cysteine

As described in section 4.2.7, H_2S plays an important role in the formation of DMTS from L-methionine. H_2S is formed during the Strecker degradation of cysteine with a diketone (Kobayashi and Fujimaki, 1965; Zheng and Ho, 1994). Therefore, the effect of L-cysteine on the formation of sulphur compounds from L-methionine was evaluated.

There was no effect of L-cysteine on the DMDS, DMTS and methional formations from L-methionine (Figures 3.66, 3.67 and 3.68). Therefore, the formation of H_2S from L-cysteine may not occur in wash distillation. However, H_2S exists in fermented wash at 9 μg/mL (Slaughter and Jordan, 1986) and is released from yeast during distillation. Therefore, H_2S concentration in fermented wash appears to determine the DMTS concentration in low wines.

4.2.9 The Formation of DMTS from DMDS

As described in section 4.2.5, with addition of CuAc2, with an increased in reaction time, DMDS concentration in the reaction mixtures decreased gradually; conversely, DMTS concentration increased. It seems that DMTS can form from DMDS. Schutte (1974) proposed that DMTS and DMS form by the disproportionation of DMDS.

To confirm this reaction, ethanol solution containing DMDS with or without copper salt was distilled. DMS and DMTS were not identified in the distillate. Although the reaction time of the model distillation was very short compared to malt whisky distilleries, the disproportionation of DMDS appeared not to occur during distillation.

4.2.10 Formation Mechanism of DMTS from DMDS from L-Methionine

Methional was identified from the decomposition products of L-methionine, similar effect of pH on the formation of DMDS and DMTS was observed and H_2S played an important role in the formation of DMTS. Therefore, the simple pathway of the formation of DMDS and DMTS from L-methionine appears to be through methional (Figure 4.4). As already described in section 4.2.5, with an increased in reaction time, DMDS concentration in the reaction mixtures decreased gradually with addition of $CuAc_2$, although DMTS concentration increased. It seems that DMTS can form by the rearrangement of DMDS which was proposed by Schutte (1974). However, as discussed in section 4.2.9, DMS and DMTS were not identified from the distillate of DMDS solution. Therefore,

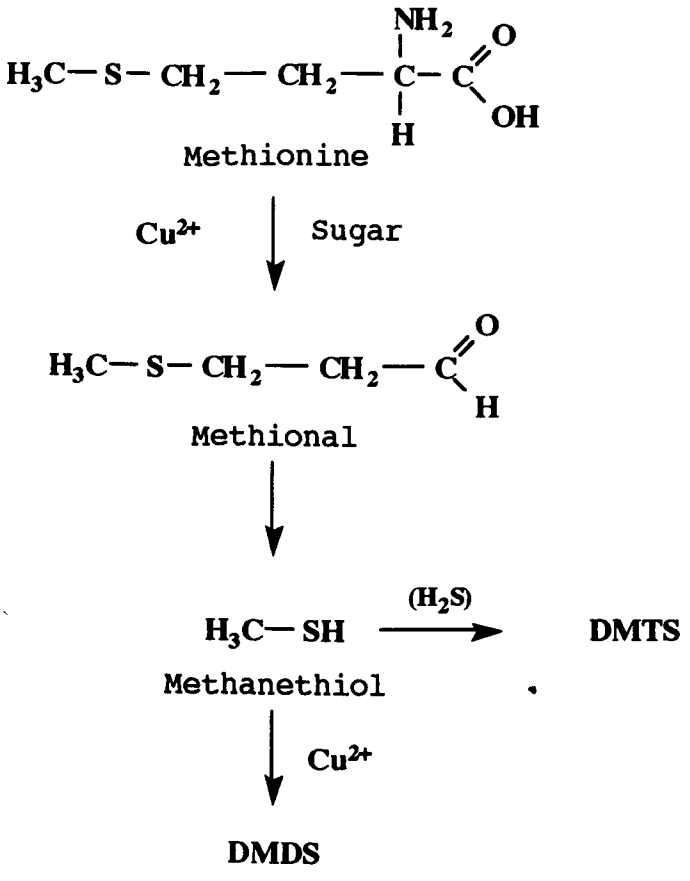

Figure 4.4 The formation mechanism of DMDS and DMTS from methionine (Yang *et al.*, 1967; Chin and Lindsay, 1994a)

there is little possibility that DMS and DMTS form from DMDS.

$CuAc_2$ increased the formation of DMTS from L-methionine, especially at high pH, despite no effect on the formation of DMTS from methional. Therefore, $CuAc_2$ appears to catalyse the formation of methional from L-methionine.

As discussed above, new evidence which suggested another pathway for the formation of DMDS and DMTS from L-methionine could not be found. Therefore, the formation mechanism of DMDS and DMTS from L-methionine appears to be through methional (Figure 4.4).

4.3 Contribution of Wash Distillation on the DMTS Concentration in Low Wines

The estimate of the contribution of wash distillation on the DMTS concentration in low wines was investigated. The methional concentration in the fermented wash is about 10 μg/mL (Masuda, 1977). From this wash, 7.5 μg/mL of DMTS can be produced maximally in distillate by the wash distillation. Although the recovery ratio of DMTS in wash distillation is unknown, the maximum recovery ratio of DMTS in spirit distillation is 70%. When this recovery ratio is applied to wash distillation, 5.3 μg/mL of DMTS in low wines originate from methional.

Methionine and maltotriose concentrations in the fermented wash are about 0.3 mM and 0.8-1.4 mM, respectively (Maemura, 1993; 1995). Therefore, if the recovery ratio of DMTS is 70% and wash distillation time is 5 h, 2.2 μg/mL of DMTS in low wines originate from methionine. Also, Beveridge (1990) reported that DMTS concentration in the fermented wash was 0.7-3.5 μg/mL. Thus, if the recovery ratio of DMTS is 70%, 1.5-7.5 μg/mL of DMTS in low wines originates from wash. It is reported that DMTS concentration in low wines was 12-30 ng/mL (Beveridge, 1990; Furusawa, 1992). Therefore, 5-50% and 25-50% of DMTS in low wines come from the fermented wash and is formed by distillation from the precursors, respectively.

The conclusion of this project can be summarised as below:

◆ Although copper is believed to remove or modify the unpleasant odour caused by sulphur compounds in whisky

industry, copper catalyses the formation of sulphur compounds during distillation.

◆ Both DMDS and DMTS were identified in the products of distillation of mixtures containing methional and L-methionine. This is the first time that DMDS and DMTS from methional and DMTS from L-methionine have been identified under the simulated conditions of a malt whisky distillation.

◆ The quantity of DMDS and DMTS from methional increased with an increase of time of heating, pH and amount of copper salt in a model distillation system. Copper salts, EDTA and sulphate ion affected the formation of these sulphur compounds.

◆ The several factors, duration of heating, reducing sugar, pH, cupric ion, aerobic conditions, oxidising agent and H_2S affected the formation of sulphur compounds from L-methionine.

◆ A mechanism of DMDS formation from methional and L-methionine was proposed whereby DMDS is formed from methanethiol; the latter was produced during distillation in the presence of methional and L-methionine.

◆ A mechanism of formation of DMTS from methional and L-methionine was proposed whereby DMTS is formed from methanethiol and H_2S.

The results of this study are important in whisky production, because the manipulation of the formation of sulphur compounds can affect the flavour of spirits. Determinants of DMDS and DMTS concentration are:

- The quantity of methional, methionine and reducing sugar in fermented wash.
- The pH of fermented wash
- The height of foam and duration of foam during wash distillation which can change the surface to volume ratio
- The duration of wash distillation and intensity of heating
- The quantity of H_2S in the fermented wash and released from yeast.
- The variety and quantity of anions in the fermented wash.

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